AD _____ (2)

CONTRACT NO: DAMD17-89-C-9119

TITLE: "SAFETY TESTING OF SEED AND VACCINES FOR DENGUE VIRUSES IN MICE, GUINEA PIGS, RABBITS AND BACTERIAL AND MYCOPLASMA CULTURE MEDIA"

PRINCIPAL INVESTIGATOR: Louis Potash, Ph.D.

PI ADDRESS: Program Resources, Inc.
7655 Old Springhouse Rd.
McLean, Virginia 22102

REPORT DATE: December 20, 1990

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick,
Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The view, opinion and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30 1986

1a. REPORT SECURITY CLASSIFICATION
Unclassified

1b. RESTRICTIVE MARKINGS

2a. SECURITY CLASSIFICATION AUTHORITY

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE

3. DISTRIBUTION / AVAILABILITY OF REPORT
Approved for public release; distribution unlimited

4 PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a NAME OF PERFORMING ORGANIZATION
Flow Laboratories, Inc.
Program Resources, Inc.

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State, and ZIP Code)
7655 Old Springhouse Rd.
McLean, VA 22102

7b. ADDRESS (City, State, and ZIP Code)

8a NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command

8b. OFFICE SYMBOL (If applicable)
SGRD-RMI-S

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
DAMD17-89-C-9119

8c. ADDRESS (City, State, and ZIP Code)
Fort Detrick
Frederick, MD 21702-5012

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO	WORK UNIT ACCESSION NO
63002A	63002D810 3M2	AC	WUDA335465

11. TITLE (Include Security Classification)
"Safety Testing of Seed and Vaccines for Dengue Viruses in Mice, Guinea Pigs, Rabbits and Bacterial and Mycoplasma Culture Media".

12. PERSONAL AUTHOR(S)
Potash, Louis

13a TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 13 Jun 89 TO 20 Dec 90	90 Dec 20	9

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	03		Safety Testing; Hepatitis A; Vaccine; Viral Seeds; RAI; Dengue-1 and Dengue-4 Production Seeds
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Preclinical safety testing of dengue virus production seeds consisted of inoculation in: 1) 5 different cell culture lines; 2) rabbits, mice (adult & newborn sucklings) and guinea pigs; and 3) bacterial fungal and mycoplasma culture media. Inocula for these tests were the crude, unclarified harvests of both control and virus fluids. For dengue-1, 3 different PDK passage levels grown in FRhL-2 cells and the same for dengue-4. All tests carried out following guidelines established by the FDA for live and inactivated virus vaccines as found in 21 CFR, Part 600 and were performed in accordance with GLP regulation A dengue-1 vaccine lot was subjected to 20 additional serial passages (total 30) in dog kidney cell cultures in attempts at further attenuation. Problems with lack of high-titered immune serum resulted in unsatisfactory findings in tissue culture purity (AGMK cells) for both viruses and in suckling mice tests with dengue-4 viruses.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS

21 ABSTRACT SECURITY CLASSIFICATION
Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL
Virginia M. Miller

22b TELEPHONE (Include Area Code)
(301) 663-7325

22c. OFFICE SYMBOL
SGRD - RMI-S

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

TABLE OF CONTENTS

I. INTRODUCTION

The Virus Vaccine Production Laboratory of Flow Laboratories, Inc. had been awarded a contract by USAMRDC to conduct preclinical testing services for Walter Reed Army Institute of Research (WRAIR) to evaluate dengue vaccines and seeds for purity, safety and potency in accordance with the technical proposal entitled "Safety Testing of Seed and Vaccines for Dengue Viruses in Mice, Guinea Pigs, Rabbits and Bacterial and Mycoplasma Culture Media". Effective Jan 1, 1990, this research, production and testing Laboratory was purchased by Program Resources, Inc. (PRI) and this contractual effort continued under an approved subcontract with PRI.

The preclinical testing services consisted of the inoculation of submitted test articles in: 1) five different cell culture lines; 2) mice (adult and new-born sucklings), guinea pigs and rabbits; and 3) bacterial, fungal and mycoplasma culture media. The inocula for these tests were crude, unclarified harvests of both control and virus fluids plus specific immune serum. All tests were carried out following guidelines established by the FDA for live and inactivated virus vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.19, 630.30 - 630.37, 630.40, 630.50 - 630.57 and 630.60 - 630.67 and were performed in accordance with Good Laboratory Practices (GLP) regulations for preclinical testing of biologicals (21 CFR, Part 58).

Although initially designed as a three (3) year contract, this effort has been brought to a close within an 13 month period with the completion of the preclinical safety testing of the following fluids: production seed pools of 3 different PDK passage levels grown in FRhL-2 cells of both dengue-1 (#45AZ5) and dengue-4 (CARIB #341750) viruses.

In addition, in an effort to attenuate a dengue-1 vaccine lot which had previously undergone 10 serial passages in dog kidney (DK) cell cultures, the virus was serially passaged 20 more times (total 30 passages) in DK cell cultures with aliquots of each passage level submitted to the COR, as directed. It was 3 of these 30 DK passage level harvests that served as the inocula for the above dengue-1 production seeds in FRhL-2 cell cultures.

II. PRECLINICAL SAFETY TESTING

Over the 13 month period, a total of 8 test articles, composed of 6 crude, unclarified virus fluids and 2 related, crude, unclarified control fluids, was safety tested. As specified in the contract workscope, these fluids were tested for:

a) microbial sterility (bacterial, fungal and mycoplasmal);
b) purity (safety) in tissue cultures (four tissue culture systems - AGMK, PHA, PRK and Flow 5000 plus the cell system in which the virus was grown - FRhL-2);
c) animal safety in rabbits, mice (adult and newborn sucklings) and guinea pigs.

The tests articles consisted of 3 dengue-1 virus production seeds plus a control fluid and 3 dengue-4 virus production seeds plus a control fluid. For the dengue-1 seeds grown in FRhL-2 cell cultures, the 10th, 20th and 27th dog kidney passage levels served as the specific inoculum. For the dengue-4 seeds grown in FRhL-2 cell cultures, the 6th, 10th and 15th dog kidney passage levels served as the specific inoculum.

DENGUE-1 VIRUS (#45AZ5)

Production Seed: PDK-10, FRhL-2/d7 of 16 Feb 90
PDK-20, FRhL-2/d7 of 16 Feb 90
PDK-27, FRhL-2/d7 of 16 Feb 90
Control Fluid ..... of 16 Feb 90

All fluids satisfactorily passed the microbial sterility tests. The results of the tissue culture purity (safety) tests were unsatisfactory only in the AGMK cell culture system with all 3 virus pools and were attributed to the failure of the supplied antiserum to completely neutralize the dengue-1 viruses. Based on a previous test with a dengue-1 virus (strain Western Pacific 1974), difficulties with the AGMK purity test were anticipated; however, pre-treatment of the primary flask cultures with immune serum 24 hours prior to inoculation with neutralized virus did not prevent dengue virus-attributed cytopathology from occurring both in the primary flasks and in the secondary tube subcultures. As expected, the tube subcultures were completely resistant to challenge with the Coxsackie A-9 virus. All virus fluids satisfactorily passed the prescribed animal safety tests. Because the AGMK purity test was unsatisfactory, these fluids were not considered to have passed all the above prescribed preclinical tests. A Phase Report detailing all of the above test results is being submitted together with this Final Report.

DENGUE-4 VIRUS (CARIB #341750)

Production Seed: PDK- 6, PRhL-2/d7 of 9 Mar 90
PDK-10, PRhL-2/d7 of 9 Mar 90
PDK-15, PRhL-2/d7 of 9 Mar 90
Control Fluid ..... of 9 Mar 90

All fluids satisfactorily passed the microbial sterility tests. The results of the tissue culture purity (safety) tests were unsatisfactory only in the AGMK cell culture system with all 3 virus pools and were attributed to the failure of the supplied antiserum to completely neutralize the dengue-4 viruses. Based on previous tissue culture purity tests with dengue viruses, difficulties with the AGMK purity test were anticipated. Dengue virus-attributed cytopathology was detected in the primary flasks as non-descript morphological changes and in the secondary tube subcultures as lytic changes. As expected, the tube subcultures were completely resistant to challenge with the Coxsackie A-9 virus. All virus fluids satisfactorily passed the prescribed animal safety tests in rabbits, adult mice and guinea pigs but results were inconclusive in suckling mice. The difficulties in the suckling mice tests, where many of the sucklings were found either dead, moribund or lethargic, were attributed to the failure of the supplied antiserum to completely neutralize the dengue-4 viruses. Because the AGMK purity test was unsatisfactory and the suckling mice tests were considered inconclusive, these fluids were not considered to have passed all the above prescribed preclinical tests. A Phase Report detailing all of the above test results is being submitted together with this Final Report.

III. SERIAL PASSAGES

Dengue Virus Type 1, Strain #45AZ5: Live-Attenuated Vaccine, Lot No. 1-82, Run 2. As an adjunct to these Preclinical Safety Tests, this laboratory continued the serial passages of this virus in dog kidney cell cultures in an effort to achieve further attenuation. Pre-screened, frozen ampules of primary dog kidney (PDK) cells (LOT 222) had been suplied by the COR. All studies were carried out in accordance with protocols submitted by the COR and included passaged control cultures. Commencing with the 10th serial passage harvest fluid produced during the previous contract, the laboratory successfully completed 20 additional serial passages using both 1st and 2nd passage DK cell cultures. Multiple 2 ml vials of each passage level (day 7 harvests of both virus infected and control cultures) were submitted to the COR.

IV. CONCLUSIONS

The preclinical safety testing of dengue virus production seeds in accordance with the specified workscope were completed. The test articles consisted of crude, unclarified harvest fluids of 3 different PDK passage levels of both dengue-1 and dengue-4 viruses grown in the FRhL-2 cell system. Due to the lack of high-titered, specific immune sera, unsatisfactory results were reported for the AGMK tissue culture purity (safety) tests on all 6 dengue virus fluids assayed. In addition, the inconclusive results obtained in the suckling mice test with the dengue-4 fluids - many of the sucklings were found either dead, moribund or lethargic - are attributed to this same lack of immune serum. It is imperative that, for any future preclinical safety testing of dengue virus fluids whether production seeds or vaccine fluids, high-titered, specific immune sera be made available so as to ensure the satisfactory completion of all the prescribed tests.

ATTACHMENT A

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 908793
Fax (301) 654-6916

December 17, 1990

Dr. Louis Potash
Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22101

Dear Dr. Potash,

Microbiological Associates, Inc. is an AAALAC accredited animal facility, and all studies are performed in accordance with the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23.

Sincerely,

Mary D. Whiteman

Mary D. Whiteman
Study Director, In Vivo Assays
Biotechnology Division

ATTACHMENT B

PRI
Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

May 14, 1991

TO: Mr. Donald Holzworth, Vice President
Dr. Louis Potash, Study Director

FROM: James R. Plautz
Sr. QA Advisor

RE: GLP Compliance Audit of Final Reports for Safety Testing of Dengue Virus Type 1 and Type 4

On April 14, 1991 a complete audit for GLP compliance (21 CFR, Part 58) was conducted for the subject final reports and their respective raw data.

Our complete findings indicate that the studies were conducted under the guidance of the referenced Standard Operating Procedures (SOPs), the variations from the SOPs had no apparent effect on study outcome, and that the final report for each study is substantiated by the raw data.

Animal safety testing was conducted and reported separately from these final reports.

James R. Plautz
May 14, 1991

APPENDIX I

Dengue-1 Virus Strain #45AZ5

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

X Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985) - (see Attachment A).

____ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45 CFR 46.

Louis G. Stark 12-20-20

PI Signature Date

TABLE OF CONTENTS

I. INTRODUCTION

The accompanying protocol is a description of the safety testing of 3 crude harvest lots of dengue virus type 1 designated as:

Dengue Virus Type 1 (#45AZ5):
PDK-10, FRhL-2/d7: PDK-20, FRhL-2/d7
and PDK-27, FRhL-2/d7 of 16 Feb 1990

Utilizing the testing procedures herein described, this fluid is considered to have not passed satisfactorily all tests for safety including purity. The detailed records with respect to passage history, pool production, and subsequent safety testing may be found in the laboratory notebooks located at:

The Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100 - (Dr. Ken Eckels)

The Experimental Virus Vaccine Production Laboratory - Suite #500 - (Flow Laboratories, Inc.) Program Resources, Inc. [PRI], McLean, VA - (Dr. Louis Potash)

All procedures performed at PRI followed Good Laboratory Practices (GLP) regulations (21 CFR, Part 58) and were carried out in accordance with the guidelines established by the FDA for live and inactivated vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.17, etc. of April 1989. These procedures are detailed in the following SOPs and recorded on the indicated VVPL Forms:

SOP No.:	500.001 -	Issued	29 Oct 1980,	Revised	13 Feb 1986
	500.002 -	"	29 Oct 1980,	"	13 Feb 1986
	500.008 -	"	13 Jan 1981,	"	3 Mar 1986
VVPL FORM	#008 -	Issued	29 Oct 1980,	Revised	3 May 1984
	016 -	"	15 Jan 1981,	"	13 July 1984
	017 -	"	16 Jan 1981,	"	13 Jan 1986
	019 -	"	8 Oct 1984		

II. SYNOPSIS

A. Crude Virus Harvests: Dengue Virus Type 1 (#45AZ5)
PDK-10, FRhL-2/d7 of 16 Feb 90
PDK-20, FRhL-2/d7 of 16 Feb 90
PDK-27, FRhL-2/d7 of 16 Feb 90

B. Safety Tests on Crude Harvest Fluids:

1. Sterility: Fluid Thioglycollate (FTM), Tryptone Soya Broth (TSB), Mycoplasma

a.	PDK-10 Virus Fluid	(47 ml)	No Growth
b.	PDK-20 Virus Fluid	(47 ml)	No Growth
c.	PDK-27 Virus Fluid	(47 ml)	No Growth
d.	Control Fluid (TCF)	(47 ml)	No Growth

2. Tissue Culture Identity and Purity (Safety): AGMK, PHA, FRhL-2, PRK, and Flow 5000.

a.	PDK-10 Virus Fluid	(25 ml)	Unsatisfactory*
b.	PDK-20 Virus Fluid	(25 ml)	Unsatisfactory*
c.	PDK-27 Virus Fluid	(25 ml)	Unsatisfactory*
d.	Control Fluid (TCF)	(25 ml)	Satisfactory

3. Animal Safety:

a. Rabbits: I.D. & S.Q. - (Appendix - B)

(1)	PDK-10 Virus Fluid	(30 ml)	Satisfactory
(2)	PDK-20 Virus Fluid	(30 ml)	Satisfactory
(3)	PDK-27 Virus Fluid	(30 ml)	Satisfactory

b. Adult Mice: I.C. & I.P - (Appendix - C)

(1)	PDK-10 Virus Fluid	(10.6 ml)	Satisfactory
(2)	PDK-20 Virus Fluid	(10.6 ml)	Satisfactory
(3)	PDK-27 Virus Fluid	(10.6 ml)	Satisfactory

---

* Test unsatisfactory only in the AGMK test system. Non-descript morphological changes observed in primary AGMK flask cultures, particularly after films were stained. All AGMK tube subcultures exhibited 2-3+ cytopathology.
Both flask and tube subcultures were negative for hemadsorption.
All tube subcultures completely inhibited the Coxsackie A-9 challenge virus.

3. Animal Safety (continued):

c.	Suckling Mice: I.C. & I.P. - (Appendix - C)		
	(1) PDK-10 Virus Fluid*	(2.2 ml)	Satisfactory
	(2) PDK-20 Virus Fluid*	(2.2 ml)	Satisfactory
	(3) PDK-27 Virus Fluid*	(2.2 ml)	Satisfactory
d.	Guinea Pigs: I.C. & I.P. - (Appendix - D)		
	(1) PDK -6 Virus Fluid	(15.3 ml)	Satisfactory
	(2) PDK-10 Virus Fluid	(15.3 ml)	Satisfactory
	(3) PDK-15 Virus Fluid	(15.3 ml)	Satisfactory

---

* Virus fluid was mixed with equal parts of a 1:5 dilution of the immune serum and incubated at 37°C for 90 minutes prior to inoculation.

III. DETAILED SUMMARY RELATING TO THE SAFETY TESTING OF THREE (3) DIFFERENT PASSAGE LEVELS OF DENGUE VIRUS TYPE 1 (#45AZ5) PRODUCTION SEEDS: PROPAGATED IN DBS-FRhL-2 CELL CULTURES

A. Inocula

In May 1990, the following frozen materials were obtained for testing from Dr. K. Eckels, Contracting Officer's Representative, Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, D.C.:

1. Dengue-1 (#45AZ5) crude, unclarified harvest fluids of 16 Feb 1990:
   a. PDK-10, FRhL2-2 (day 7 harvest)..... 20 x 10 ml vials
   b. PDK-20, FRhL2-2 (day 7 harvest)..... 20 x 10 ml vials
   c. PDK-27, FRhL2-2 (day 7 harvest)..... 20 x 10 ml vials
   d. Control Fluids ..... 4 x 25 ml vials

2. Dengue-1 Antiserum: Jamaica HMAF of 4/17/73 ... 1 x 8 ml

On arrival in this laboratory, the virus and control fluids were stored at -70°C, or below, and the antiserum at -20°C, or below.

B. Safety Testing Procedures and Results on the Crude, Unclarified Harvest Fluids (SOP No.: 500.008)

1. Microbial Sterility - (VVPL FORM #011)

Aliquots of the bulk frozen fluids were thawed and tested for microbial sterility as follows:

a. Fluid Thioglycollate Medium - FTM - (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 culture tubes were included as uninoculated controls. All cultures were vortex mixed and incubated at 32°C (± 2°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.

b. Tryptone Soya Broth - TSB - (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 cultures were included as uninoculated controls. All cultures were vortex mixed and incubated at 22°C (± 2°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.

The results of the above described Microbial Sterility Assays are summarized in Table I.

c. Mycoplasma Sterility: These assays were performed by PRI's Mycoplasma Testing Laboratory and included both the routine PPLO agar and broth assays and the specific test for the detection of M. hyorhinis. Samples (1 x 2 ml and 1 x 25 ml) of the 3 crude virus fluids and of the 1 control fluid were submitted for testing. All samples were reported to be negative for mycoplasmas. A copy of this report is appended to this Protocol - (Appendix A - 1, 2, 3 & 4).

2. Identity in Tissue Culture (Serum-Neutralization) -

No attempt was made to identity the crude virus pools in tissue cultures.

3. Purity (Safety) in Tissue Cultures - (VVPL FORM #016)

a. Tissue Cultures: All flask and roller tube cell cultures were prepared by contract personnel. Cultures were maintained on Medium MEM containing 5 to 10% fetal bovine serum (heat-inactivated) plus antibiotics: gentamicin @ 100 mcg/ml; neomycin @ 50 mcg/ml; and amphotericin B (I.V.) @ 2.5 mcg/ml. Cultures were inoculated, refed and subpassaged as indicated below. The following tissue culture systems were utilized:

(1) Tertiary African Green Monkey Kidney (AGMK) ..... MEM + 5% serum
(2) Primary Human Amnion (PHA) ..... MEM + 10% serum
(3) Fetal Rhesus Lung (FRhL-2) ..... MEM + 5% serum
(4) Primary Rabbit Kidney (PRK) ..... MEM + 5% serum
(5) Whole Human Embryo Fibroblast (Flow 5000) ..... MEM + 5% serum

b. General Testing Procedures

(1) Crude Virus Fluids

(a) Primary Flask Cultures: Equal 5 ml volumes of the bulk crude virus fluids and of a 1:5 dilution of the rabbit immune serum (Den-1, Jamaica HMAF) were well mixed and incubated at 37°C (water bath) for 90 minutes. Due to the small volume of antiserum available, only 5 ml of each the virus fluids were tested per tissue culture system wherein 1 x 75 cm^2 flask per tissue culture system was inoculated with 10 ml of these serum-virus mixtures. These flasks were pre-treated 24 hours earlier with 0.5 ml of undiluted immune serum and overlayed with 25 ml of maintenance medium. Cultures were incubated at 35°C (37°C for PHA) for 14 days with periodic microscopic examination for any signs of CPE and/or cellular degradation. When necessary to maintain the integrity of the cell films, cultures were refed with 35 ml of fresh medium.

(b) Secondary Tube Subcultures: On the 14th day of incubation, the primary cultures were re-examined microscopically and the fluids harvested individually and treated with the specific immune serum - 0.1 ml per harvest. In addition, to each individual harvest was added: 0.1 ml gentamicin (50 mg/ml); 1 ml penicillin-streptomycin solution (5000 units/ml and 5000 mcg/ml, respectively); and 10% of 10X SPG* (v/v).

---

* 10X SPG: sucrose, 2.13 M; KH_2PO_4, 0.038 M; K_2HPO_4, 0.072 M; monosodium glutamate, 0.049 M.

Following mixing, the fluids were incubated at room temperature for 60 min. and then subpassed into homologous roller tube cultures - 0.5 ml of each harvest into each of 20 tubes. The remainder of the harvest fluids was saved and stored at -75°C, or below. All primary cultures were tested for hemadsorption by the addition of 0.1% guinea pig RBC (in PBS) and incubation at 4°C for a minimum of 30 minutes. All cultures were negative for hemadsorption.

Tube cultures (refed with 2 ml of maintenance medium prior to inoculation) were incubated at 35°C (37°C for PHA) for 13-14 additional days. When necessary to maintain the integrity of the cell films, cultures were refed with 2 ml of fresh medium. Cultures were examined microscopically at periodic intervals and at the end of the incubation period for any signs of CPE. After final examination, tubes were divided - depending on the specific cell system - for additional testing:

AGMK, PHA, FRhL-2 and Flow 5000 Tube Cultures: These were divided into 3 groups as follows:

1/4th tested for the presence of hemadsorbing agents,
1/4th fixed and stained with a solution of 5% glutaraldehyde + 1:10 giemsa stain and examined microscopically for any CPE,
1/2 Challenged with Coxsackie A-9 virus (0.2 ml per tube at dilutions noted in the tables) for the detection of non-CPE producing agents and/or latent agents via the interference phenomenon.

PRK Tube Cultures: These were equally divided into 2 groups:

1/2 tested for the presence of hemadsorbing agents,
1/2 fixed and stained with the glutaraldehyde-giemsa stain solution and examined microscopically for any CPE.

No challenge studies were carried out with the Coxsackie A-9 virus since this virus does not produce any discernible CPE in this tissue culture system.

(2) Crude Control Fluid

A single 75 cm^2 flask per tissue culture system was inoculated with 10 ml of crude control fluid. Cultures were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included.

(3) Uninoculated Cell Lot Controls

Two x 75 cm^2 flasks per tissue culture system were included as uninoculated cell lot controls and were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included. In addition, an appropriate number of uninoculated roller tube cultures were included as cell lot controls for the secondary tube subcultures.

In all challenge studies, 1 to 4 culture tubes per set were left unchallenged to serve as controls to the challenge virus.

The results of these in vitro Tissue Culture Purity (Safety) tests are summarized in Tables II-A through -E.

4. Animal Safety Tests

Due to the dismantling of Flow's Animal Facility during December 1989, all animal studies were performed by Microbiological Associates, Inc. The inocula for these animal studies were the three crude virus suspensions

a. Adult Rabbits - MBA Studies #ZA356.005101, #ZA357.005101 and #ZA358.005101 - these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix - B.

b. Adult and Suckling Mice - MBA Studies #ZA356.005100, #ZA357.005100 and #ZA358.005100 - all three tests in both adult mice and in suckling mice were reported to be satisfactory and copies of these Final Reports may be found in Appendix - C.

c. Adult Guinea Pigs - MBA Studies #ZA356.005102, #ZA357.005102 and #ZA358.005102 - these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix - D.

Table I. Microbial Sterility Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools

Culture Medium	No.	Vol. per culture (ml)	Temperature	Date On Test	Date Off Test	Results
Fluid Thioglycollate						
(FTM) LOT VVPL-#030	10	-----	32°C (±2°C)	11/12/90	12/03/90	No Growth
PDK-10 Virus Fluid	10	1.0		|	|	No Growth
PDK-20 Virus Fluid	10	1.0		|	|	No Growth
PDK-27 Virus Fluid	10	1.0		|	|	No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth
Tryptone Soya Broth						
(TSB) LOT VVPL #030	10	-----	22°C (±2°C)	11/12/90	12/0[illegible]	[illegible]Growth
PDK-10 Virus Fluid	10	1.0		|	|	[illegible] Growth
PDK-20 Virus Fluid	10	1.0		|	|	No Growth
PDK-27 Virus Fluid	10	1.0		|	|	No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools

A. Tertiary African Green Monkey Kidney (AGMK)

Material Tested	Initial Flasks Lot #1618 (#2129, p4)) Day 14			0.5 ml per tube Passage #1 Lot # 1656 (2129, p3) Day 14 + 14 = 28			Coxsackie A-9 Challenge*			
	CPE	Hads	Stain	CPE	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	**		**	***		***				
PDK-10 Virus/Serum Mixture	1/1	0/1	1/1	20/20	0/ 5	5/ 5	0/2	0/2	0/2	0/2
PDK-20 Virus/Serum Mixture	1/1	0/1	1/1	20/20	0/ 5	5/ 5	0/2	0/2	0/2	0/2
PDK-27 Virus/Serum Mixture	1/1	0/1	1/1	20/20	0/ 5	5/ 5	0/2	0/2	0/2	0/2
Control Fluid (TCF)	0/1	0/1	0/1	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control - (1)	0/2	0/2	0/2	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	8/8	4/8

* Coxsackie A-9 Challenge Results based on a 6-day incubation at 35°C.
Prior to challenge, all tubes refed with 2 ml of fresh medium
Complete inhibition of Coxsackie A-9 challenge virus by virus/serum mixture series.

** Non-descript cytopatholgy initially observed on day 10 and confirmed on staining on day 14 for all 3 virus/serum inoculated flasks only.

*** On day 20 (days 14 + 6), all tubes inoculated with harvests from virus/serum inoculated flasks exhibited cytopathology which progressed to 3-4+ by day 28 (days 14 + 14). This cytopathology, confirmed on staining, was attributed to dengue virus breakthroughs. Islands of cells remained which proved to be resistant to the Coxsackie A-9 challenge virus.

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45A.5) Production Seed Pools

B. Primary Human Amnion (PHA)

Material Tested	Initial Flasks Lot # 1541 Day: 14			0.5 ml per tube Passage #1 Lot # 1639 Day: 14 + 14 = 28			Coxsackie A-9 Challenge*			
	CPE**	Hads	Stain	CPE	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
PDK-20 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	2/2
PDK-27 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	7/8	1/8

* Coxsackie A-9 Challenge Results based on a 4-day incubation at 37°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

** On day 7, all flasks were refed with 35 ml of fresh medium.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools

C. Fetal Rhesus Lung (FRhL-2)

Material Tested	Initial Flasks Lot # 1610 p21 Day 14			0.5 ml per tube Passage #1 Lot # 1697 p24 Day 14 + 14 = 28			Coxsackie A-9 Challenge*			
	CPE	Hads	Stain	CPE	Hads	Stain	10^{-2}	10^{-3}	10^{-4}	10^{-5}
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
PDK-20 Virus/Serum Mixture	0/1	0/1	ND	0/19	0/ 5	0/ 5	2/2	2/2	2/2	0/2
PDK-27 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	3/4
Control - (2)				0/30	0/12	0/12	8/8	8/8	8/8	1/8

* Coxsackie A-9 Challenge Results based on a 3-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45A25) Production Seed Pools

D. Primary Rabbit Kidney (PRK)

	Initial Flasks			0.5 ml per tube Passage #1		
	Lot # 1650			Lot # 1693		
	Day: 14			Day: 14 + 14 = 28		
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/10	0/10
PDK-20 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/10	0/10
PDK-27 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/10	0/10
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/10	0/10
Control - (1)	0/2	0/2	ND	0/40	0/20	0/20
Control - (2)				0/24	0/12	0/12

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-1 Virus (#45AZ5) Production Seed Pools

E. Whole Human Embryo Fibroblasts (Flow 5000)

Material Tested	Initial Flasks Lot # 1630 p18 Day 14			0.5 ml per tube Passage #1 Lot # 1669 p20 Day 14 + 14 = 28			Coxsackie A-9 Challenge*			
	CPE	Hads	Stain	CPE	Hads	Stain	10^{-2}	10^{-3}	10^{-4}	10^{-5}
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	2/2
PDK-20 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
PDK-27 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	2/2
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	1/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	5/8	2/8

* Coxsackie A-9 Challenge Results based on a 5-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

ND = Not done

PRI Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

8 August, 1990.

To: Dr. Louis Potash.

From: Jim Quartey. JQ

Subject: Mycoplasma Testing. (Charge # 807)

This letter is to inform you that, the eight (8) samples listed below which you had submitted for the detection of Mycoplasma hyorhinis using the direct immunofluorescence staining and for the detection of Mycoplasma in general using the DNA Hoechst stain and Agar testing were found to be negative.

a. Dengue-1 (#45AZ5) Production Seed of 16 Feb 90:

1. PDK-10, FRhL-2/d7.
2. PDK-20, FRhL-2/d7.
3. PDK-27, FRhL-2/d7.
4. Control Fluid.

b. Dengue-4 (#341750) Production Seed of 9 Mar 90:

1. PDK-6, FRhL-2/d7
2. PDK-10,FRhL-2/d7
3. PDK -15,FRhL-2/d7
4. Control Fluid.

Source: Dr. Rosen ; Date Received: 7/6/90

Approved by _____
Effective Date: 5 Sept. 1982

Date: 7/7/90 Set up by: Jim Cooley

Page 1 of 1

Projected Final Reading: 7/31/90

CHARGE 807.

Billing Date: _____ ; Billing Number: _____

8 samples.

Identification Number	#	Preliminary Reading 7/16 Agar 1	Agar 2	BTS-7 1	BTS-7 2	Hoechst 1	Hoechst 2	Final 7/31 Agar 1	Agar 2	Sub day	Sub 1	Sub 2	Notes	Re
Negative Control		0		0		0		0						C
Positive Control		+		+		+		+						A
DENGUE-1 (#45AZ5) PDK-10, FRhL-2/27	197	0		0		0		0						C
PDK-20, FRhL-2/27	198	0		0		0		0						C
PDK-27, FRhL-2/27	199	0		0		0		0						C
CONTROL FLUID	200	0		0		0		0						C
DENGUE-4 (#341750) PDK-6, FRhL-2/27	201	0		0		0		0						C
PDK-10, FRhL-2/27	202	0		0		0		0						C
PDK-15, FRhL-2/27	203	0		0		0		0						C
CONTROL FLUID	204	0		0		0		0						C
Read by:	xxxxxxx	JJ		JJ		JJ		JJ						C
Date:	xxxxxxx	7/16		7/25		7/25		7/31						7/31/90

KEY: + = Postive
0 = Negative
? = Questionable
BC = Bacterial contamination

Plate Lot No. 900523 Hoechst Lot No. 044486

BTG Lot 094 Planted by Jim C

MYCOPLASMA TEST RECORD SHEET

Culture Medium	LOT #	No. ml Tested		Date		Results
		Aerobic	Anaerobic	On Test	Off Test	
DENGUE-1 (#45AZ5) Virus Fluid - LOT # PDK-10, FRhL-2/27. MYC# 197						
PPLO Agar	900523	.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth	900503	25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
DENGUE-1 (#45AZ5) ~~Virus~~ Control Fluid - LOT # PDK-20, FRhL-2/27. MYC# 198						
PPLO Agar		.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth		25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE

Positive Control (+): M. arginini Negative Control (-): FB 29101 C070

Date: 8/8/90 Signed: Jim Darley

MYCOPLASMA TEST RECORD SHEET

Culture Medium	LOT #	No. ml Tested Aerobic	No. ml Tested Anaerobic	Date On Test	Date Off Test	Results
DENGUE-1 (#45AZ5) Virus Fluid - LOT # PDK 27, FRhL-2/d7. Myc#199.						
PPLO Agar	900523	.2	.2	7/9/90	7/24/90	NEGATIVE.
PPLO Broth	900503	25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
DENGUE-1 (#45AZ5) Control Fluid - LOT # Myc # 200.						
PPLO Agar		.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth		25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE

Positive Control (+): M. orale Negative Control (-): FB 2910/COTO

Date: 8/8/90 Signed: Terri Cowley

APPENDIX

B

ANIMAL SAFETY TEST IN ADULT RABBITS

ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA356.005101

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Rabbits

B. Study Number: ZA356.005101

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Rabbits, four females, SPF NZW, 1.5 - 2.5 kg.
Source: Buckshire Corp.
P.O. Box 155
Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1.

Note: In error, animals were inoculated by intraocular route. This deviation from the protocol did not affect the outcome of this assay.

The rabbits were observed for 28 days for clinical signs of illness or distress.

3. Animal Husbandry

a. Rabbits were fed certified rabbit chow ad libitum and water was supplied via water bottles, ad libitum.

b. Rabbit's cages were changed weekly.

c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman

Mary D. Whiteman
Study Director

9/27/90

Date

ZA356.005101

TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q. I.O.	1.0 ml 9.0 ml 0.03 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation
S.Q. = Subcutaneous Inoculation
I.O. = Intraocular Inoculation

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

	RABBITS
Test Article	3/3[a]
Uninoculated Control	1/1[b]

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.
[b] Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.)
Rabbit	Test Article	1	Normal		
		2	Normal		
		3	Normal		
	Uninoculated Control	4	Normal		

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA356.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/03/90 - 08/03/90, TO STUDY DIR 08/03/90, TO MGMT 08/06/90
PHASES: ADMINISTRATION OF TEST ARTICLE TO TEST SYSTEM

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton — 9-28-90

Ed Warburton
Quality Assurance Unit

Date

ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA357.005101

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7.

ZA357.005101

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Rabbits

B. Study Number: ZA357.005101

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Rabbits, four females, SPF NZW, 1.5 - 2.5 kg.
Source: Buckshire Corp.
P.O. Box 155
Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

3. Animal Husbandry

a. Rabbits were fed certified rabbit chow ad libitum and water was supplied via water bottles, ad libitum.

b. Rabbit's cages were changed weekly.

c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/27/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation
S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

	RABBITS
Test Article	3/3[a]
Uninoculated Control	1/1[b]

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.
[b] Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.)
Rabbit	Test Article	5	Normal		
		6	Normal		
		7	Normal		
	Uninoculated Control	4	Normal		

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA357.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/03/90 - 08/03/90, TO STUDY DIR 08/03/90, TO MGMT 08/06/90
PHASES: ADMINISTRATION OF TEST ARTICLE TO TEST SYSTEM

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton — 9-28-90

Ed Warburton
Quality Assurance Unit

Date

ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA358.005101

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Rabbits

B. Study Number: ZA358.005101

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Rabbits, four females, SPF NZW, 1.5 - 2.5 kg.
Source: Buckshire Corp.
P.O. Box 155
Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

3. Animal Husbandry

a. Rabbits were fed certified rabbit chow ad libitum and water was supplied via water bottles, ad libitum.

b. Rabbit's cages were changed weekly.

c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/27/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation
S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

	RABBITS
Test Article	3/3[a]
Uninoculated Control	1/1[b]

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.
[b] Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.)
Rabbit	Test Article	8	Normal		
		9	Normal		
		10	Normal		
	Uninoculated Control	4	Normal		

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA358.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/23/90 - 07/23/90, TO STUDY DIR 07/23/90, TO MGMT 07/23/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton — 9-28-90

Ed Warburton
Quality Assurance Unit

Date

APPENDIX

C

ANIMAL SAFETY TEST IN

ADULT MICE AND SUCKLING MICE

ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA356.005100

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue, after 14 days, into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of viral contamination due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was observed.

ZA356.005100

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Mice and Suckling Mice

B. Study Number: ZA356.005100

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. The test article was received frozen. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)FBR, three adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms
Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)FBR, four adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms
Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams.
Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: 5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

With approval of the sponsor, a previously thawed sample which was frozen back and stored at -70°C was utilized in the inoculation of the suckling mouse portion of the assay on 07/24/90. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-1 Jamaica HMAF, 4-17-78 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay on 07/26/90.

A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated

according to Table 1 and were then observed every working day for 14 days for clinical signs. Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervial dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

3. Animal Husbandry

   a. All animals were fed the following diet ad libitum:

      Mice - autoclavable chow.

   b. Water was supplied ad libitum via fresh apples (disinfected).

   c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

   d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and all test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C.

In the blind passage, all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice and all of the suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of viral contamination due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 8/31/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

*SAA = Same as above
i.c. = Intracranial
i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

		Suckling Mice[b]	
	Adult Mice[a]	Primary Inoculation	Blind Passage
Test Article	20/20	20/20	20/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control[c]			10/10

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.

[b] In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

[c] In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.
Adult Mice	Test Article	10501	Normal		
		10502	Normal		
		10503	Normal		
		10504	Normal		
		10505	Normal		
		10506	Normal		
		10507	Normal		
		10508	Normal		
		10509	Normal		
		10510	Normal		
		10511	Normal		
		10512	Normal		
		10513	Normal		
		10514	Normal		
		10515	Normal		
		10516	Normal		
		10517	Normal		
		10518	Normal		
		10519	Normal		
		10520	Normal		
	Uninoc-ulated Control	10521	Normal		
		10522	Normal		
		10523	Normal		
		10524	Normal		
		10525	Normal		
		10526	Normal		
		10527	Normal		
		10528	Normal		
		10529	Normal		
		10530	Normal		

TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
Suckling[b] Mice (Primary Inoculation)	Test Article	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Uninoculated Control	SM3 (10)	Normal		
(Blind Passage)	Test Article Homogenate	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Uninoculated Control Homogenate	SM3 (10)	Normal		
	Uninoculated Control	SM4 (10)	Normal		

[a] Ten suckling mice inoculated per cage.
[b] Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA356.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90
PHASES: OBSERVATION OF ANIMALS FOR CLINICAL SIGNS

INSPECT ON 08/30/90 - 08/30/90, TO STUDY DIR 08/30/90, TO MGMT 08/31/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan — 8/31/90

Joan M. McGowan
Quality Assurance Unit

Date

ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA357.005100

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue, after 14 days, into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of viral contamination due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was observed.

ZA357.005100

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Mice and Suckling Mice

B. Study Number: ZA357.005100

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. The test article was received frozen. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)FBR, three adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms
Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)FBR, four adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms
Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams.
Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: 5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

With approval of the sponsor, a previously thawed sample which was frozen back and stored at -70°C was utilized in the inoculation of the suckling mouse portion of the assay on 07/24/90. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-1 Jamaica HMAF, 4-17-78 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay on 07/26/90.

A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every

working day for 14 days for clinical signs. Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

3. Animal Husbandry

   a. All animals were fed the following diet ad libitum:

      Mice - autoclavable chow.

   b. Water was supplied ad libitum via fresh apples (disinfected).

   c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

   d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C.

In the blind passage all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice, and nineteen of the twenty suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article homogenate inoculated suckling mice was missing and presumed cannibalized day 3 post-inoculation.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of viral contamination due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman 8/31/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

*SAA = Same as above
i.c. = Intracranial
i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

	Adult Mice[a]	Suckling Mice[b] Primary Inoculation	Suckling Mice[b] Blind Passage
Test Article	20/20	20/20	19/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control[c]			10/10

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.

[b] In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

[c] In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.
Adult Mice	Test Article	10531	Normal		
		10532	Normal		
		10533	Normal		
		10534	Normal		
		10535	Normal		
		10536	Normal		
		10537	Normal		
		10538	Normal		
		10539	Normal		
		10540	Normal		
		10541	Normal		
		10542	Normal		
		10543	Normal		
		10544	Normal		
		10545	Normal		
		10546	Normal		
		10547	Normal		
		10548	Normal		
		10549	Normal		
		10550	Normal		
	Uninoc-ulated Control	10521	Normal		
		10522	Normal		
		10523	Normal		
		10524	Normal		
		10525	Normal		
		10526	Normal		
		10527	Normal		
		10528	Normal		
		10529	Normal		
		10530	Normal		

TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
Suckling[b] Mice (Primary Inoculation)	Test Article	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Uninoculated Control	SM3 (10)	Normal		
(Blind Passage)	Test Article Homogenate	SM1 (10)	Normal		
		(9)[c]	-	3	3
		SM2 (10)	Normal		
	Uninoculated Control Homogenate	SM3 (10)	Normal		
	Uninoculated Control	SM4 (10)	Normal		

[a] Ten suckling mice inoculated per cage.
[b] Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.
[c] One suckling mouse missing and presumed cannibalized.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA357.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90
PHASES: OBSERVATION OF ANIMALS FOR CLINICAL SIGNS

INSPECT ON 08/30/90 - 08/30/90, TO STUDY DIR 08/30/90, TO MGMT 08/31/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan — 8/31/90

Joan M. McGowan
Quality Assurance Unit

Date

ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA358.005100

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue, after 14 days, into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of viral contamination due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was observed.

ZA358.005100

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Mice and Suckling Mice

B. Study Number: ZA358.005100

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. The test article was received frozen. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)FBR, three adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms
Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)FBR, four adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms
Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females,
Body Weight range: 15-20 grams.
Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: 5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

With approval of the sponsor, a previously thawed sample which was frozen back and stored at -70°C was utilized in the inoculation of the suckling mouse portion of the assay on 07/24/90. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-1 Jamaica HMAF, 4-17-78 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay on 07/26/90.

A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs.

Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

3. Animal Husbandry

   a. All animals were fed the following diet ad libitum:

      Mice - autoclavable chow.

   b. Water was supplied ad libitum via fresh apples (disinfected).

   c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

   d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and all test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C.

In the blind passage, all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice and all of the suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of viral contamination due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 8/31/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

*SAA = Same as above
i.c. = Intracranial
i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

	Adult Mice[a]	Suckling Mice[b] Primary Inoculation	Suckling Mice[b] Blind Passage
Test Article	20/20	20/20	20/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control[c]			10/10

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.

[b] In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

[c] In the blind passage of the suckling mouse portion of the assay, an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.
Adult Mice	Test Article	10551	Normal		
		10552	Normal		
		10553	Normal		
		10554	Normal		
		10555	Normal		
		10556	Normal		
		10557	Normal		
		10558	Normal		
		10559	Normal		
		10560	Normal		
		10561	Normal		
		10562	Normal		
		10563	Normal		
		10564	Normal		
		10565	Normal		
		10566	Normal		
		10567	Normal		
		10568	Normal		
		10569	Normal		
		10570	Normal		
	Uninoc-ulated Control	10521	Normal		
		10522	Normal		
		10523	Normal		
		10524	Normal		
		10525	Normal		
		10526	Normal		
		10527	Normal		
		10528	Normal		
		10529	Normal		
		10530	Normal		

TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
Suckling[b] Mice (Primary Inoculation)	Test Article	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Uninoculated Control	SM3 (10)	Normal		
(Blind Passage)	Test Article Homogenate	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Uninoculated Control Homogenate	SM3 (10)	Normal		
	Uninoculated Control	SM4 (10)	Normal		

[a] Ten suckling mice inoculated per cage.
[b] Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA358.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/21/90
PHASES: OBSERVATION OF ANIMALS FOR CLINICAL SIGNS

INSPECT ON 08/30/90 - 08/30/90, TO STUDY DIR 08/30/90, TO MGMT 08/31/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan Mc Gowan — 8/31/90

Joan M. McGowan — Date
Quality Assurance Unit

APPENDIX

D

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA356.005102

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this test is to detect the presence of adventitious agent(s) which might be present in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7.

ZA356.005102

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Guinea Pigs

B. Study Number: ZA356.005102

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Guinea Pigs
Hartley Albino
6 adult females,
Body weight range: 350-400 g
Source: Hazleton Research Animals
Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

3. Animal Husbandry

a. Animals were fed Ralston Purina Certified Guinea Pig Chow.

b. Water was supplied ad libitum via water bottles. Water was disinfected with 7 ppm chlorine.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post-Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

IV. RESULTS

All of the uninoculated control guinea pigs and two of the three test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. One of the test article inoculated guinea pigs (#10401) was noted to have a noticeably decreased amount of feces production on day 15 post-inoculation, but appeared otherwise normal. By day 19 post-inoculation, feces production appeared normal and the animal remained normal and healthy for the duration of the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through 42 post-inoculation. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the control or test article guinea pigs. (See Pathology report in Appendix.)

V. CONCLUSIONS

No evidence of contamination with adventitious agents due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/12/90

Mary D. Whiteman
Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK=10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.	Range of Body Temp in °C D-21 to D-42
Guinea Pig	Test Article	10401	-[a]	15		38.0 - 38.5
		10402	Normal			37.9 - 38.7
		10403	Normal			37.9 - 38.4
	Uninoc-ulated Control	10404	Normal			38.0 - 38.5
		10405	Normal			38.2 - 39.0
		10406	Normal			38.0 - 38.9

[a] Animal was noted to have reduced feces production, but appeared otherwise normal. Feces production was normal by day 19 and appeared normal for the duration of the 42 day observation period.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA356.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan McGowan — 9/14/90

Joan M. McGowan
Quality Assurance Unit

Date

VIII. APPENDIX

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

PATHOLOGY REPORT

CAHS-2190 **DATE RECEIVED:** 08/23/90

SOURCE: Biotech Services **DATE NECROPSIED:** 08/23/90
ZA356.005102 ZA357.005102
ZA358.005102 ZA359.005102 **DATE REPORTED:** 09/06/90
ZA360.005102 ZA361.005102

SPECIES: Guinea Pig
Results of gross examination according to SOP #865.201.

ZA356.005102

2190-1 (10401) Test Article 563.9 g
Gross: No lesions found

2190-2 (10402) Test Article 684.2 g
Gross: No lesions found

2190-3 (10403) Test Article 614.8 g
Gross: No lesions found

2190-4 (10404) Control 614.4 g
Gross: No lesions found

2190-5 (10405) Control 673.3 g
Gross: No lesions found

2190-6 (10406) Control 646.2 g
Gross: No lesions found

ZA357.005102

2190-7 (10407) Test Article 629.0 g
Gross: No lesions found

2190-8 (10408) Test Article 583.6 g
Gross: No lesions found

2190-9 (10409) Test Article 528.1 g
Gross: No lesions found

ZA358.005102

2190-10 (10410) Test Article 636.3 g
Gross: No lesions found

2190-11 (10411) Test Article 490.5 g
Gross: Retroperitoneal abscess (4x6 cm) and peritonitis

2190-12 (10412) Test Article 595.5 g
Gross: No lesions found

ZA359.005102

2190-13 (10421) Test Article 605.3 g
Gross: No lesions found

2190-14 (10422) Test Article 631.8 g
Gross: No lesions found

2190-15 (10423) Test Article 547.4 g
Gross: No lesions found

2190-16 (10424) Control 651.5 g
Gross: No lesions fc ıd

2190-17 (10425) Control 623.6 g
Gross: No lesions found

2190-18 (10426) Control 615.9 g
Gross: No lesions found

ZA360.005102

2190-19 (10427) Test Article 561.8 g
Gross: No lesions found

2190-20 (10428) Test Article 594.6 g
Gross: No lesions found

2190-21 (10429) Test Article 598.4 g
Gross: No lesions found

ZA361.005102

2190-22 (10430) Test Article 559.8 g
Gross: No lesions found

2190-23 (10431) Test Article 547.9 g
Gross: No lesions found

2190-24 (10432) Test Article 624.8 g
Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA357.005102

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

ZA357.005102

TABLE OF CONTENTS

SUMMARY

The purpose of this test is to detect the presence of adventitious agent(s) which might be present, in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed, due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7.

ZA357.005102

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Guinea Pigs

B. Study Number: ZA357.005102

C. Test Article: Dengue-1 (≡45AZ5) Prod Seed, PDK-20, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Guinea Pigs
Hartley Albino
6 adult females,
Body weight range: 350-400 g
Source: Hazleton Research Animals
Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

3. Animal Husbandry

a. Animals were fed Ralston Purina Certified Guinea Pig Chow.

b. Water was supplied ad libitum via water bottles. Water was disinfected with 7 ppm chlorine.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post-Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through day 42 post-inoculation.

At examination, on day 42, for gross pathology, no lesions were found in the uninoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/12/90

Mary D. Whiteman
Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-20, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.	Range of Body Temp in °C D-21 to D-42
Guinea Pig	Test Article	10407	Normal			38.0 - 38.8
		10408	Normal			38.1 - 38.9
		10409	Normal			37.9 - 38.7
	Uninoc-ulated Control	10404	Normal			38.0 - 38.5
		10405	Normal			38.2 - 39.0
		10406	Normal			38.0 - 38.9

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA357.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/24/90
PHASES: EXAM. OF ABDOMINAL AND THORACIC VISCERA AT DAY 42 POST-INOCULATION FOR OBVIOUS OR SUGGESTIVE ABNORMALITIES

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan
Quality Assurance Unit

9/14/90
Date

VIII. APPENDIX

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

PATHOLOGY REPORT

CAHS-2190 **DATE RECEIVED:** 08/23/90

SOURCE: Biotech Services **DATE NECROPSIED:** 08/23/90
ZA356.005102 ZA357.005102
ZA358.005102 ZA359.005102 **DATE REPORTED:** 09/06/90
ZA360.005102 ZA361.005102

SPECIES: Guinea Pig
Results of gross examination according to SOP #865.201.

ZA356.005102

2190-1 (10401) Test Article 563.9 g
Gross: No lesions found

2190-2 (10402) Test Article 684.2 g
Gross: No lesions found

2190-3 (10403) Test Article 614.8 g
Gross: No lesions found

2190-4 (10404) Control 614.4 g
Gross: No lesions found

2190-5 (10405) Control 673.3 g
Gross: No lesions found

2190-6 (10406) Control 646.2 g
Gross: No lesions found

ZA357.005102

2190-7 (10407) Test Article 629.0 g
Gross: No lesions found

2190-8 (10408) Test Article 583.6 g
Gross: No lesions found

2190-9 (10409) Test Article 528.1 g
Gross: No lesions found

ZA358.005102

2190-10 (10410) Test Article 636.3 g
Gross: No lesions found

2190-11 (10411) Test Article 490.5 g
Gross: Retroperitoneal abscess (4x6 cm) and peritonitis

2190-12 (10412) Test Article 595.5 g
Gross: No lesions found

ZA359.005102

2190-13 (10421) Test Article 605.3 g
Gross: No lesions found

2190-14 (10422) Test Article 631.8 g
Gross: No lesions found

2190-15 (10423) Test Article 547.4 g
Gross: No lesions found

2190-16 (10424) Control 651.5 g
Gross: No lesions found

2190-17 (10425) Control 623.6 g
Gross: No lesions found

2190-18 (10426) Control 615.9 g
Gross: No lesions found

ZA360.005102

2190-19 (10427) Test Article 561.8 g
Gross: No lesions found

2190-20 (10428) Test Article 594.6 g
Gross: No lesions found

2190-21 (10429) Test Article 598.4 g
Gross: No lesions found

ZA361.005102

2190-22 (10430)	Test Article	559.8 g
Gross: No lesions found		
2190-23 (10431)	Test Article	547.9 g
Gross: No lesions found		
2190-24 (10432)	Test Article	624.8 g
Gross: No lesions found		

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA358.005102

Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this test is to detect the presence of adventitious agent(s) which might be present, in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed, due to the test article Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7.

ZA358.005102

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Guinea Pigs

B. Study Number: ZA358.005102

C. Test Article: Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Guinea Pigs
Hartley Albino
6 adult females,
Body weight range: 350-400 g

Source: Hazleton Research Animals
Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

3. Animal Husbandry

a. Animals were fed Ralston Purina Certified Guinea Pig Chow.

b. Water was supplied ad libitum via water bottles. Water was disinfected with 7 ppm chlorine.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post-Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

IV. RESULTS

All of the uninoculated control guinea pigs and two of the three test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. One of the test article inoculated guinea pigs (10411) was noted to appear lethargic with a rough hair coat and decreased fluid intake on day 35 post-inoculation. By day 38 post-inoculation, the animal appeared normal. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infection during the 21 day recording period from day 21 through day 42. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in any of the uninoculated control animals and no lesions were found in two of the three test article inoculated animals. One of the test article inoculated animals (10411) was found to have a (4 x 6 cm) retroperitoneal abscess and peritonitis. The abscess was attributed to a rectal perforation caused by insertion of a temperature measuring probe and was considered to be a common lesion in guinea pigs in which a probe is inserted on multiple occasions. (See Pathology Report in Appendix.)

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/13/90

Mary D. Whiteman
Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-1 (#45AZ5) Prod Seed, PDK-27, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.	Range of Body Temp in °C D-21 to D-42
Guinea Pig	Test Article	10410	Normal			37.8 - 38.8
		10411	-[a]	35		38.0 - 38.8
		10412	Normal			37.9 - 38.6
	Uninoc-ulated Control	10404	Normal			38.0 - 38.5
		10405	Normal			38.2 - 39.0
		10406	Normal			38.0 - 38.9

[a] Animal had a rough hair coat and appeared lethargic with a decreased fluid intake day 35 post-inoculation. Animal appeared normal by day 38 post-inoculation

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA358.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90
PHASES: RECTAL TEMPERATURE DETERMINATION

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan McGowan — 9/14/90

Joan M. McGowan
Quality Assurance Unit

Date

VIII. APPENDIX

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

PATHOLOGY REPORT

CARS-2190 **DATE RECEIVED:** 08/23/90

SOURCE: Biotech Services **DATE NECROPSIED:** 08/23/90
ZA356.005102 ZA357.005102
ZA358.005102 ZA359.005102 **DATE REPORTED:** 09/06/90
ZA360.005102 ZA361.005102

SPECIES: Guinea Pig
Results of gross examination according to SOP #865.201.

ZA356.005102

2190-1 (10401) Test Article 563.9 g
Gross: No lesions found

2190-2 (10402) Test Article 684.2 g
Gross: No lesions found

2190-3 (10403) Test Article 614.8 g
Gross: No lesions found

2190-4 (10404) Control 614.4 g
Gross: No lesions found

2190-5 (10405) Control 673.3 g
Gross: No lesions found

2190-6 (10406) Control 646.2 g
Gross: No lesions found

ZA357.005102

2190-7 (10407) Test Article 629.0 g
Gross: No lesions found

2190-8 (10408) Test Article 583.6 g
Gross: No lesions found

2190-9 (10409) Test Article 528.1 g
Gross: No lesions found

ZA358.005102

2190-10 (10410) Test Article 636.3 g
Gross: No lesions found

2190-11 (10411) Test Article 490.5 g
Gross: Retroperitoneal abscess (4x6 cm) and peritonitis

2190-12 (10412) Test Article 595.5 g
Gross: No lesions found

ZA359.005102

2190-13 (10421) Test Article 605.3 g
Gross: No lesions found

2190-14 (10422) Test Article 631.8 g
Gross: No lesions found

2190-15 (10423) Test Article 547.4 g
Gross: No lesions found

2190-16 (10424) Control 651.5 g
Gross: No lesions found

2190-17 (10425) Control 623.6 g
Gross: No lesions found

2190-18 (10426) Control 615.9 g
Gross: No lesions found

ZA360.005102

2190-19 (10427) Test Article 561.8 g
Gross: No lesions found

2190-20 (10428) Test Article 594.6 g
Gross: No lesions found

2190-21 (10429) Test Article 598.4 g
Gross: No lesions found

ZA361.005102

2190-22 (10430) Test Article 559.8 g
Gross: No lesions found

2190-23 (10431) Test Article 547.9 g
Gross: No lesions found

2190-24 (10432) Test Article 624.8 g
Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

ATTACHMENT A

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 908793
Fax (301) 654-6916

December 17, 1990

Dr. Louis Potash
Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22101

Dear Dr. Potash,

Microbiological Associates, Inc. is an AAALAC accredited animal facility, and all studies are performed in accordance with the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23.

Sincerely,

Mary D. Whiteman

Mary D. Whiteman
Study Director, In Vivo Assays
Biotechnology Division

ATTACHMENT B

PRI

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

May 14, 1991

TO: Mr. Donald Holzworth, Vice President
Dr. Louis Potash, Study Director

FROM: James R. Plautz
Sr. QA Advisor

RE: GLP Compliance Audit of Final Reports for Safety Testing of Dengue Virus Type 1 and Type 4

On April 14, 1991 a complete audit for GLP compliance (21 CFR, Part 58) was conducted for the subject final reports and their respective raw data.

Our complete findings indicate that the studies were conducted under the guidance of the referenced Standard Operating Procedures (SOPs), the variations from the SOPs had no apparent effect on study outcome, and that the final report for each study is substantiated by the raw data.

Animal safety testing was conducted and reported separately from these final reports.

James R. Plautz
May 14, 1991

APPENDIX II

Dengue-4 Virus Strain Carib 341750

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____Where copyrighted material is quoted, permission has been obtained to use such material.

_____Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

X Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985) - (see Attachment A).

_____For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45 CFR 46.

Louis G. Stark 12-20-90

PI Signature Date

TABLE OF CONTENTS

I. INTRODUCTION

The accompanying protocol is a description of the safety testing of 3 crude harvest lots of dengue virus type 4 designated as:

Dengue Virus Type 4 (Carib 341750):
PDK-6, FRhL-2/d7: PDK-10, FRhL-2/d7
and PDK-15, FRhL-2/d7 of 9 March 1990

Utilizing the testing procedures herein described, this fluid is considered to have not passed satisfactorily all tests for safety including purity. The detailed records with respect to passage history, pool production, and subsequent safety testing may be found in the laboratory notebooks located at:

The Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100 - (Dr. Ken Eckels)

The Experimental Virus Vaccine Production Laboratory - Suite #500 - (Flow Laboratories, Inc.) Program Resources, Inc. [PRI], McLean, VA - (Dr. Louis Potash)

All procedures performed at PRI followed Good Laboratory Practices (GLP) regulations (21 CFR, Part 58) and were carried out in accordance with the guidelines established by the FDA for live and inactivated vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.17, etc. of April 1989. These procedures are detailed in the following SOPs and recorded on the indicated VVPL Forms:

SOP No.:	500.001 -	Issued	29 Oct 1980,	Revised	18 Feb 1986
	500.002 -	"	29 Oct 1980,	"	18 Feb 1986
	500.008 -	"	13 Jan 1981,	"	3 Mar 1986
VVPL FORM	#008 -	Issued	29 Oct 1980,	Revised	3 May 1984
	016 -	"	15 Jan 1981,	"	13 July 1984
	017 -	"	16 Jan 1981,	"	13 Jan 1986
	019 -	"	8 Oct 1984		

II. SYNOPSIS

A. Crude Virus Harvests: Dengue Virus Type 4 (Carib 341750)
PDK- 6, FRhL-2/d7 of 9 Mar 90
PDK-10, FRhL-2/d7 of 9 Mar 90
PDK-15, FRhL-2/d7 of 9 Mar 90

B. Safety Tests on Crude Harvest Fluids:

1. Sterility: Fluid Thioglycollate (FTM), Tryptone Soya Broth (TSB), Mycoplasma

a.	PDK- 6 Virus Fluid	(47 ml)	No Growth
b.	PDK-10 Virus Fluid	(47 ml)	No Growth
c.	PDK-15 Virus Fluid	(47 ml)	No Growth
d.	Control Fluid (TCF)	(47 ml)	No Growth

2. Tissue Culture Identity and Purity (Safety): AGMK, PHA, FRhL-2, PRK, and Flow 5000.

a.	PDK- 6 Virus Fluid	(25 ml)	Unsatisfactory*
b.	PDK-10 Virus Fluid	(25 ml)	Unsatisfactory*
c.	PDK-15 Virus Fluid	(25 ml)	Unsatisfactory*
d.	Control Fluid (TCF)	(25 ml)	Satisfactory

3. Animal Safety:

a. Rabbits: I.D. & S.Q. - (Appendix - B)

(1)	PDK- 6 Virus Fluid	(30 ml)	Satisfactory
(2)	PDK-10 Virus Fluid	(30 ml)	Satisfactory
(3)	PDK-15 Virus Fluid	(30 ml)	Satisfactory

b. Adult Mice: I.C. & I.P - (Appendix - C)

(1)	PDK -6 Virus Fluid	(10.6 ml)	Satisfactory
(2)	PDK-10 Virus Fluid	(10.6 ml)	Satisfactory
(3)	PDK-15 Virus Fluid	(10.6 ml)	Satisfactory

---

* Test unsatisfactory only in the AGMK test system. Non-descript morphological changes observed in primary AGMK flask cultures, particularly after films were stained. All AGMK tube subcultures exhibited varying degrees of cytopathology ranging from 1-3+. Both flask and tube subcultures were negative for hemadsorption. All tube subcultures completely inhibited the Coxsackie A-9 challenge virus.

3. Animal Safety (continued):

c. Suckling Mice: I.C. & I.P. - (Appendix - C)

(1)	PDK- 6 Virus Fluid*	(2.2 ml)	Satisfactory
(2)	PDK-10 Virus Fluid*	(2.2 ml)	Inconclusive**
(3)	PDK-15 Virus Fluid*	(2.2 ml)	Inconclusive***

d. Guinea Pigs: I.C. & I.P. - (Appendix - D)

(1)	PDK -6 Virus Fluid	(15.3 ml)	Satisfactory
(2)	PDK-10 Virus Fluid	(15.3 ml)	Satisfactory
(3)	PDK-15 Virus Fluid	(15.3 ml)	Satisfactory

---

* Virus fluid was mixed with equal parts of a 1:5 dilution of the immune serum and incubated at 37°C for 90 minutes prior to inoculation.

** Although all of the 20 sucklings appeared normal and survived the initial 14-day incubation period, only 9 of the 20 sucklings inoculated with their emulsified tissue survived the final 14-day blind passage with 2 lethargic and with hunched postures.

*** Only 6 of 15 inoculated sucklings appeared normal at the end of the initial 14-day incubation period and were emulsified for blind passage. Of the other 10 original sucklings, 1 was apparently cannibalized, 4 were found dead (marked autolysis on necrosy & histopathology) and 5 were found moribund and were homogenized for subpassage. None of the 20 sucklings inoculated with the 'normal' homogenate survived the final 14-day blind passage and, of the 20 sucklings inoculated with the moribund homogenate, 10 survived but of these 2 were moribund and 1 was lethargic and runted.

III. DETAILED SUMMARY RELATING TO THE SAFETY TESTING OF THREE (3) DIFFERENT PASSAGE LEVELS OF DENGUE VIRUS TYPE 4 (CARIB 341750) PRODUCTION SEEDS: PROPAGATED IN DBS-FRhL-2 CELL CULTURES

A. Inocula

In May 1990, the following frozen materials were obtained for testing from Dr. K. Eckels, Contracting Officer's Representative, Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, D.C.:

1. Dengue-4 (Carib 341750) crude, unclarified harvest fluids of 9 Mar 90:
   a. PDK- 6, FRhL2-2 (day 7 harvest)..... 20 x 10 ml vials
   b. PDK-10, FRhL2-2 (day 7 harvest)..... 20 x 10 ml vials
   c. PDK-15, FRhL2-2 (day 7 harvest)..... 20 x 10 ml vials
   d. Control Fluids ..... 4 x 25 ml vials

2. Dengue-4 Antiserum: 814669 CAREC, SM-5 OF 11/21/82 ... 1 x 8 ml

On arrival in this laboratory, the virus and control fluids were stored at -70°C, or below, and the antiserum at -20°C, or below.

B. Safety Testing Procedures and Results on the Crude, Unclarified Harvest Fluids (SOP No.: 500.008)

1. Microbial Sterility - (VVPL FORM #011)

Aliquots of the bulk frozen fluids were thawed and tested for microbial sterility as follows:

a. Fluid Thioglycollate Medium - FTM - (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 culture tubes were included as uninoculated controls. All cultures were vortex mixed and incubated at 32°C (± 2°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.

b. Tryptone Soya Broth - TSB - (LOT VVPL #030): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus fluids and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid. An additional 10 cultures were included as uninoculated controls. All cultures were vortex mixed and incubated at 22°C (± 2°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 50 culture tubes.

The results of the above described Microbial Sterility Assays are summarized in Table I.

c. Mycoplasma Sterility: These assays were performed by PRI's Mycoplasma Testing Laboratory and included both the routine PPLO agar and broth assays and the specific test for the detection of M. hyorhinis. Samples (1 x 2 ml and 1 x 25 ml) of the 3 crude virus fluids and of the 1 control fluid were submitted for testing. All samples were reported to be negative for mycoplasmas. A copy of this report is appended to this Protocol - (Appendix A - 1, 2, 3 & 4).

2. Identity in Tissue Culture (Serum-Neutralization) -

No attempt was made to identity the crude virus pools in tissue cultures.

3. Purity (Safety) in Tissue Cultures - (VVPL FORM #016)

a. Tissue Cultures: All flask and roller tube cell cultures were prepared by contract personnel. Cultures were maintained on Medium MEM containing 5 to 10% fetal bovine serum (heat-inactivated) plus antibiotics: gentamicin @ 100 mcg/ml; neomycin @ 50 mcg/ml; and amphotericin B (I.V.) @ 2.5 mcg/ml. Cultures were inoculated, refed and subpassaged as indicated below. The following tissue culture systems were utilized:

(1) Tertiary African Green Monkey Kidney (AGMK) ..... MEM + 5% serum
(2) Primary Human Amnion (PHA) ..... MEM + 10% serum
(3) Fetal Rhesus Lung (FRhL-2) ..... MEM + 5% serum
(4) Primary Rabbit Kidney (PRK) ..... MEM + 5% serum
(5) Whole Human Embryo Fibroblast (Flow 5000) ..... MEM + 5% serum

b. General Testing Procedures

(1) Crude Virus Fluids

(a) Primary Flask Cultures: Equal 5 ml volumes of the bulk crude virus fluids and of a 1:10 dilution of the rabbit immune serum (Den-4, CAREC 814669, smb 5) were well mixed and incubated at 37°C (water bath) for 90 minutes. Due to the small volume of antiserum available, only 5 ml of each the virus fluids were tested per tissue culture system wherein 1 x 75 cm^2 flask per tissue culture system was inoculated with 10 ml of these serum-virus mixtures. Flasks contained approximately 25 ml of maintenance medium at the time of inoculation. Cultures were incubated at 35°C (37°C for PHA) for 14 days with periodic microscopic examination for any signs of CPE and/or cellular degradation. When necessary to maintain the integrity of the cell films, cultures were refed with 35 ml of fresh medium.

(b) Secondary Tube Subcultures: On the 14th day of incubation, the primary cultures were re-examined microscopically and the fluids harvested individually and treated with the specific immune serum - 0.1 ml per harvest. In addition, to each individual harvest was added: 0.1 ml gentamicin (50 mg/ml); 1 ml penicillin-streptomycin solution (5000 units/ml and 5000 mcg/ml, respectively); and 10% of 10X SPG* (v/v).

---

* 10X SPG: sucrose, 2.18 M; KH_2PO_4, 0.038 M; K_2HPO_4, 0.072 M; monosodium glutamate, 0.049 M.

Following mixing, the fluids were incubated at room temperature for 60 min. and then subpassed into homologous roller tube cultures - 0.5 ml of each harvest into each of 20 tubes. The remainder of the harvest fluids was saved and stored at -75°C, or below. All primary cultures were tested for hemadsorption by the addition of 0.1% guinea pig RBC (in PBS) and incubation at 4°C for a minimum of 30 minutes. All cultures were negative for hemadsorption.

Tube cultures (refed with 2 ml of maintenance medium prior to inoculation) were incubated at 35°C (37°C for PHA) for 13-14 additional days. When necessary to maintain the integrity of the cell films, cultures were refed with 2 ml of fresh medium. Cultures were examined microscopically at periodic intervals and at the end of the incubation period for any signs of CPE. After final examination, tubes were divided - depending on the specific cell system - for additional testing:

AGMK, PHA, FRhL-2 and Flow 5000 Tube Cultures: These were divided into 3 groups as follows:

1/4th tested for the presence of hemadsorbing agents,
1/4th fixed and stained with a solution of 5% glutaraldehyde + 1:10 giemsa stain and examined microscopically for any CPE,
1/2 Challenged with Coxsackie A-9 virus (0.2 ml per tube at dilutions noted in the tables) for the detection of non-CPE producing agents and/or latent agents via the interference phenomenon.

PRK Tube Cultures: These were equally divided into 2 groups:

1/2 tested for the presence of hemadsorbing agents,
1/2 fixed and stained with the glutaraldehyde-giemsa stain solution and examined microscopically for any CPE.

No challenge studies were carried out with the Coxsackie A-9 virus since this virus does not produce any discernible CPE in this tissue culture system.

(2) Crude Control Fluid

A single 75 cm^2 flask per tissue culture system was inoculated with 10 ml of crude control fluid. Cultures were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included.

(3) Uninoculated Cell Lot Controls

Two x 75 cm^2 flasks per tissue culture system were included as uninoculated cell lot controls and were handled in a manner similar to that described above for the crude virus fluid except that immune serum was not included. In addition, an appropriate number of uninoculated roller tube cultures were included as cell lot controls for the secondary tube subcultures.

In all challenge studies, 1 to 4 culture tubes per set were left unchallenged to serve as controls to the challenge virus.

The results of these in vitro Tissue Culture Purity (Safety) tests are summarized in Tables II-A through -E.

4. Animal Safety Tests

Due to the dismantling of Flow's Animal Facility during December 1989, all animal studies were performed by Microbiological Associates, Inc. The inocula for these animal studies were the three crude virus suspensions

a. Adult Rabbits - MBA Studies #ZA359.005101, #ZA360.005101 and #ZA361.005101 - these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix - B.

b. Adult and Suckling Mice - MBA Studies #ZA359.005100, #ZA360.005100 and #ZA361.005100 - all three tests in adult mice were reported to be satisfactory. However, of the three sucking mice tests, only Study #ZA359.005100 (PDK-6, FRhL-2/d7 inoculum) was reported to be satisfactory, whereas the tests in the other 2 studies (#ZA360.005100 and #ZA361.005100 - PDK-10 & PDK-15) were reported to be inconclusive due to the lethal effect of the test articles. Copies of these Final Reports may be found in Appendix - C.

c. Adult Guinea Pigs - MBA Studies #ZA359.005102, #ZA360.005102 and #ZA361.005102 - these tests were reported to be satisfactory and copies of these Final Reports may be found in Appendix - D.

Table I. Microbial Sterility Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools

Culture Medium	No.	Vol. per culture (ml)	Temperature	Date On Test	Date Off Test	Results
Fluid Thioglycollate						
(FTM) LOT VVPL-#030	10	-----	32°C (±2°C)	11/12/90	12/03/90	No Growth
PDK- 6 Virus Fluid	10	1.0		|	|	No Growth
PDK-10 Virus Fluid	10	1.0		|	|	No Growth
PDK-15 Virus Fluid	10	1.0		|	|	No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth
Tryptone Soya Broth						
(TSB) LOT VVPL #030	10	-----	22°C (±2°C)	11/12/90	12/03/90	No Growth
PDK- 6 Virus Fluid	10	1.0		|	|	No Growth
PDK-10 Virus Fluid	10	1.0		|	|	No Growth
PDK-15 Virus Fluid	10	1.0		|	|	No Growth
Control Fluid	10	1.0		11/12/90	12/03/90	No Growth

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools

A. Tertiary African Green Monkey Kidney (AGMK)

	Initial Flasks			0.5 ml per tube Passage #1						
	Lot #1618 (#2129, p4))			Lot # 1656 (2129, p3)						
	Day 14			Day 14 + 14 = 28						
							Coxsackie A-9 Challenge*			
Material Tested	CPE	Hads	Stain**	CPE#	Hads	Stain***	10^{-3}	10^{-4}	10^{-5}	10^{-6}
PDK- 6 Virus/Serum Mixture	0/1	0/1	1/1	20/20	0/ 5	5/ 5	0/2	0/2	0/2	0/2
PDK-10 Virus/Serum Mixture	0/1	0/1	1/1	20/20	0/ 5	5/ 5	0/2	0/2	0/2	0/2
PDK-15 Virus/Serum Mixture	0/1	0/1	1/1	20/20	0/ 5	5/ 5	0/2	0/2	0/2	0/2
Control Fluid (TCF)	0/1	0/1	0/1	0/20	0/ 5	0/ 5	2/2	2/2	1/2	0/2
Control - (1)	0/2	0/2	0/2	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	8/8	4/8

* Coxsackie A-9 Challenge Results based on a 6-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium Complete inhibition of Coxsackie A-9 challenge virus by virus/serum mixture series.

** Stained flasks revealed non-descript CPE in virus/serum inoculated flasks only.

All tubes inoculated with harvests from virus/serum inoculated flasks exhibited cytopathology on day 20 (days 14 + 6) which was attributed to dengue virus breakthroughs.

*** Staining of tubes confirmed the varying degrees of cytopathology: PDK-6 = PDK-15 < PDK-10.

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools

B. Primary Human Amnion (PHA)

Material Tested	Initial Flasks Lot # 1541 Day: 14			0.5 ml per tube Passage #1 Lot # 1639 Day: 14 + 14 = 28			Coxsackie A-9 Challenge*			
	** CPE	Hads	Stain	CPE	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
PDK- 6 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	1/2	0/2
PDK-15 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	0/2	0/2
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	7/8	1/8

* Coxsackie A-9 Challenge Results based on a 4-day incubation at 37°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

** On day 7, all flasks were refed with 35 ml of fresh medium.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools

C. Fetal Rhesus Lung (FRhL-2)

Material Tested	Initial Flasks Lot # 1610 p21 Day 14			0.5 ml per tube Passage #1 Lot # 1687 p24 Day 14 + 14 = 28			Coxsackie A-9 Challenge*			
	CPE	Hads	Stain	CPE	Hads	Stain	10^{-2}	10^{-3}	10^{-4}	10^{-5}
PDK- 6 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
PDK-15 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	0/2
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	3/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	8/8	1/8

* Coxsackie A-9 Challenge Results based on a 3-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools

D. Primary Rabbit Kidney (PRK)

	Initial Flasks			0.5 ml per tube Passage #1		
	Lot # 1650			Lot # 1693		
	Day: 14			Day: 14 + 14 = 28		
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain
PDK -6 Virus/Serum Mixture	0/1*	0/1	ND	0/20	0/10	0/10
PDK-10 Virus/Serum Mixture	0/1*	0/1	ND	0/20	0/10	0/10
PDK-15 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/10	0/10
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/10	0/10
Control - (1)	0/2	0/2	ND	0/40	0/20	0/20
Control - (2)				0/24	0/12	0/12

* Commencing on day 10, focal area of vacuolation observed in these flasks only. Whatever caused these morphological changes was not subpassaged into the tube subcultures.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Dengue-4 Virus (Carib 341750) Production Seed Pools

E. Whole Human Embryo Fibroblasts (Flow 5000)

Material Tested	Initial Flasks Lot # 1630 p18 Day 14			0.5 ml per tube Passage #1 Lot # 1669 p20 Day 14 + 14 = 28			Coxsackie A-9 Challenge*			
	CPE	Hads	Stain	CPE	Hads	Stain	10^{-2}	10^{-3}	10^{-4}	10^{-5}
PDK- 6 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
PDK-10 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	2/2
PDK-15 Virus/Serum Mixture	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	2/2	1/2
Control Fluid (TCF)	0/1	0/1	ND	0/20	0/ 5	0/ 5	2/2	2/2	1/2	1/2
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	1/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	5/8	2/8

* Coxsackie A-9 Challenge Results based on a 5-day incubation at 35°C. Prior to challenge, all tubes refed with 2 ml of fresh medium.

ND = Not done

PRI Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

8 August, 1990.

To: Dr. Louis Potash.

From: Jim Quartey. JQ

Subject: Mycoplasma Testing. (Charge # 807)

This letter is to inform you that, the eight (8) samples listed below which you had submitted for the detection of Mycoplasma hyorhinis using the direct immunofluorescence staining and for the detection of Mycoplasma in general using the DNA Hoechst stain and Agar testing were found to be negative.

a. Dengue-1 (#45AZ5) Production Seed of 16 Feb 90:

1. PDK-10, FRhL-2/d7.
2. PDK-20, FRhL-2/d7.
3. PDK-27, FRhL-2/d7.
4. Control Fluid.

b. Dengue-4 (#341750) Production Seed of 9 Mar 90:

1. PDK-6, FRhL-2/d7
2. PDK-10, FRhL-2/d7
3. PDK -15, FRhL-2/d7
4. Control Fluid.

Source: Dr. Porter; Data Received: 7/6/90

Approved by [illegible]
Effective Date: 5 Sept. 1982

Date: 7/9/90 Set up by: Jim Cooley

Page 1 of 1

Projected Final Reading: 7/31/90

CHARGE 807.
8 samples.

Billing Date: _____; Billing Number: _____

Identification Number	#	Preliminary Reading 7/16 Agar 1	Agar 2	BTS-7 1	BTS-7 2	Hoechst 1	Hoechst 2	Final 7/31 Agar 1	Agar 2	Sub day	Sub 1	Sub 2	Notes	R
Negative Control		0		0		0		0						
Positive Control		+		+		+		+						
DENGUE-1 (#45AZ5) PDK-10, FRhL-2/d7	197	0		0		0		0						
PDK-20, FRhL-2/d7	198	0		0		0		0						
PDK-27, FRhL-2/d7	199	0		0		0		0						
CONTROL FLUID	200	0		0		0		0						
DENGUE-4 (#341750) PDK-6, FRhL-2/d7	201	0		0		0		6						
PDK-10, FRhL-2/d7	202	0		0		0		0						
PDK-15, FRhL-2/d7	203	0		0		0		0						
CONTROL FLUID	204	0		0		0		0						
Read by:	xxxxxxx xxxxxxx	J						J						
Date:	xxxxxxx xxxxxxx	7/16		7/25		7/25		7/31						7/31/90

KEY: + = Postive
0 = Negative
? = Questionable
BC = Bacterial contumination

Plate Lot No. 900523 Hoechst Lot No. 044486

3T6 Lot 794 Planted by Jim C

MYCOPLASMA TEST RECORD SHEET

Culture Medium	LOT #	No. ml Tested Aerobic	No. ml Tested Anaerobic	Date On Test	Date Off Test	Results
DENGUE-4 (#341750) Virus Fluid - LOT # PDK-6, FRhL-2/27. Myc# 201						
PPLO Agar	900523	.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth	900503	25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/40	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
DENGUE-4 (#341750) ~~Control~~ Virus Fluid - LOT # PDK-10, FRhL-2/27. Myc# 202						
PPLO Agar		.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth		25.0	25 0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	
to Agar		.2	.2			

Positive Control (+): M. orale Negative Control (-): FB 29101 C070

Date: 8/8/90 Signed: Jim Cawley

MYCOPLASMA TEST RECORD SHEET

Culture Medium	LOT #	No. ml Tested		Date		Results
		Aerobic	Anaerobic	On Test	Off Test	
DENGUE-4 (#341750) Virus Fluid - LOT # PDK-15, FRhL-2/27. Myc #203						
PPLO Agar	900523	.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth	900503	25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
DENGUE-4 (#341750) Control Fluid - LOT # Myc # 204.						
PPLO Agar		.2	.2	7/9/90	7/24/90	NEGATIVE
PPLO Broth		25.0	25.0			NEGATIVE
D 5 Subpass to Broth		25.0	25.0	7/16/90	7/31/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	25.0	7/19/90	8/3/90	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	25.0	7/24/90	8/8/90	NEGATIVE
to Agar		.2	.2			NEGATIVE

Positive Control (+): M. arginini Negative Control (-): FB 2910/C070

Date: 8/8/90 Signed: Jim Quarley

APPENDIX

B

ANIMAL SAFETY TEST IN ADULT RABBITS

ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA359.005101

Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Rabbits

B. Study Number: ZA359.005101

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Rabbits, four females, SPF NZW, 1.5 - 2.5 kg.
Source: Buckshire Corp.
P.O. Box 155
Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

3. Animal Husbandry

a. Rabbits were fed certified rabbit chow ad libitum and water was supplied via water bottles, ad libitum.

b. Rabbit's cages were changed weekly.

c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/27/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation
S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

	RABBITS
Test Article	3/3[a]
Uninoculated Control	1/1[b]

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.
[b] Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.)
Rabbit	Test Article	11	Normal		
		12	Normal		
		13	Normal		
	Uninoculated Control	4	Normal		

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA359.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton — 9-28-90

Ed Warburton
Quality Assurance Unit

Date

ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA360.005101

Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article pre-clarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Rabbits

B. Study Number: ZA360.005101

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Rabbits, four females, SPF NZW, 1.5 - 2.5 kg.
Source: Buckshire Corp.
P.O. Box 155
Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

3. Animal Husbandry

a. Rabbits were fed certified rabbit chow ad libitum and water was supplied via water bottles, ad libitum.

b. Rabbit's cages were changed weekly.

c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK10, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman
Mary D. Whiteman
Study Director

9/28/90
Date

ZA360.005101

TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation
S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

	RABBITS
Test Article	3/3[a]
Uninoculated Control	1/1[b]

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.
[b] Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.)
Rabbit	Test Article	14	Normal		
		15	Normal		
		16	Normal		
	Uninoculated Control	4	Normal		

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA360.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton — 9-28-90

Ed Warburton
Quality Assurance Unit

Date

ANIMAL SAFETY TEST IN ADULT RABBITS

Study No.: ZA361.005101

Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9000 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article preclarified bulk live virus vaccine and/or fluids, other than the specific virus in the product. The test article was inoculated into adult rabbits.

No evidence of contamination with adventitious agent(s) was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilized inoculation of adult rabbits. The test is performed as described in CFR Title 21, Section 630.16.

Adult rabbits are utilized in this assay to detect possible contamination of the test article with B-virus or other adventitious agent(s) including other Simian agents, adenovirus(es), etc. which might be present in the test article. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Rabbits

B. Study Number: ZA361.005101

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was received at Microbiological Associates, Inc. 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Rabbits, four females, SPF NZW, 1.5 - 2.5 kg.
Source: Buckshire Corp.
P.O. Box 155
Perkasie, PA 18944

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate Study Director: Janet Luczak, M.T. (ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 08/03/90

3. Lab Completion Date: 08/31/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect inapparent adventitious agent(s) which might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each rabbit was housed individually. Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Each rabbit was identified by a unique number tattooed on it's ear. The rabbit's number was recorded on the cage card.

The rabbits were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The rabbits were observed for 28 days for clinical signs of illness or distress.

3. Animal Husbandry

a. Rabbits were fed certified rabbit chow ad libitum and water was supplied via water bottles, ad libitum.

b. Rabbit's cages were changed weekly.

c. Animal facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All rabbits inoculated with the test article and the uninoculated control rabbit remained normal and healthy for the 28 day observation period.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman
Mary D. Whiteman
Study Director

9/27/90
Date

ZA361.005101

TABLE 1

Group	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.D. S.Q.	1.0 ml 9.0 ml	Test Article	Observe for Illness
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	None	SAA

SAA = Same As Above
I.D. = Intradermal Inoculation
S.Q. = Subcutaneous Inoculation

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

	RABBITS
Test Article	3/3[a]
Uninoculated Control	1/1[b]

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.
[b] Number of surviving, healthy animals after 28 days/Number of animals on lab initiation date.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.)
Rabbit	Test Article	17	Normal		
		18	Normal		
		19	Normal		
	Uninoculated Control	4	Normal		

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT RABBITS

Study Number: ZA361.005101

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/16/90 - 08/16/90, TO STUDY DIR 08/16/90, TO MGMT 08/21/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/21/90 - 09/24/90, TO STUDY DIR 09/24/90, TO MGMT 09/28/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Ed Warburton — 9-28-90

Ed Warburton
Quality Assurance Unit

Date

APPENDIX

C

ANIMAL SAFETY TEST IN

ADULT MICE AND SUCKLING MICE

ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA359.005100

Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus vaccines and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of contamination with adventitious agents due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was observed.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Mice and Suckling Mice

B. Study Number: ZA359.005100

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Mice

Suckling litters (Primary Inoculation): Tac(SW)fBR, three adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms, Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)fBR, four adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms, Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females,
Body Weight range: 15-20 grams.
Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

With approval of the sponsor, a previously thawed test article sample which was frozen back and stored at -70°C ± 10°C was utilized in the inoculation of the suckling mouse portion of the assay. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-4 #814669 Carec, SM5 of 11-21-82 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay.

A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs. Fourteen days post-inoculation, all surviving

suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

3. Animal Husbandry

   a. All animals were fed the following diet ad libitum:

      Mice - autoclavable chow.

   b. Water was supplied ad libitum via fresh apples.

   c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

   d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All uninoculated control suckling mice and test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. The surviving mice of each group were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at $-70^{o}C \pm 10^{o}C$.

In the blind passage all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice, and nineteen of the twenty suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article homogenate inoculated suckling mice was missing and presumed cannibalized day four post-inoculation.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agents was observed due to the test article, Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 10/23/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

SAA = Same as above
i.c. = Intracranial
i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

	Adult Mice[a]	Suckling Mice[b] Primary Inoculation	Suckling Mice[b] Blind Passage
Test Article	20/20	20/20	19/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control[c]			10/10

[a] Number of surviving, healthy animals after 28 days/Number of animals inoculated.

[b] In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

[c] In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.
Adult Mice	Test Article	10601	Normal		
		10602	Normal		
		10603	Normal		
		10604	Normal		
		10605	Normal		
		10606	Normal		
		10607	Normal		
		10608	Normal		
		10609	Normal		
		10610	Normal		
		10611	Normal		
		10612	Normal		
		10613	Normal		
		10614	Normal		
		10615	Normal		
		10616	Normal		
		10617	Normal		
		10618	Normal		
		10619	Normal		
		10620	Normal		
	Uninoc-ulated Control	10621	Normal		
		10622	Normal		
		10623	Normal		
		10624	Normal		
		10625	Normal		
		10626	Normal		
		10627	Normal		
		10628	Normal		
		10629	Normal		
		10630	Normal		

TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
Suckling[b] Mice (Primary Inoculation)	Test Article	SM1 (10)	Normal		
		SM2 (10)	Normal		
	Uninoculated Control	SM3 (10)	Normal		
(Blind Passage)	Test Article Homogenate	SM1 (10)	Normal		
		(9)[c]		4	4
		SM2 (10)	Normal		
	Uninoculated Control Homogenate	SM3 (10)	Normal		
	Uninoculated Control	SM4 (10)	Normal		

[a] Ten suckling mice inoculated per cage.

[b] Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.

[c] One suckling mouse was missing and presumed cannibalized.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA359.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 10/17/90 - 10/17/90, TO STUDY DIR 10/17/90, TO MGMT 10/25/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Dana H. Hamblen
Quality Assurance Unit

10-25-90
Date

ANIMAL SAFETY TEST IN
ADULT MICE AND SUCKLING MICE

Study No.: ZA360.005100

Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus vaccines and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of contamination with adventitious agents due to the test article Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive due to the lethal effect of the specific virus in the product, on the suckling mice.

ZA360.005100

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent virus(es). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Mice and Suckling Mice

B. Study Number: ZA360.005100

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)fBR, three adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms, Germantown, New York

Suckling litters (Blind Passage): Tac:(SW)fBR, four adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms, Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females,
Body Weight range: 15-20 grams.
Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/23/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

With approval of the sponsor, a previously thawed test article sample which was frozen back and stored at -70°C ± 10°C was utilized in the inoculation of the suckling mouse portion of the assay. In addition, at the request of the sponsor, in the suckling mouse portion of the assay, 1.3 ml of the test article was combined with 1.3 ml of sponsor supplied antisera Den-4 #814669 Carec, SM5 of 11-21-82 and heated at 37°C for 90 minutes, prior to inoculation of the suckling mice. The remaining untreated sample was again frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay.

A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs.

Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamicin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

3. Animal Husbandry

   a. All animals were fed the following diet ad libitum:

      Mice - autoclavable chow.

   b. Water was supplied ad libitum via fresh apples.

   c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

   d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All of the uninoculated control suckling mice and nineteen of the twenty test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article inoculated suckling mice appeared lethargic and had a swollen head day 14 post-inoculation, prior to sacrifice of all surviving mice of

each group. The surviving mice were homogenized and the homogenate of each group was passaged into a new group of suckling mice. The remainder of the homogenates was frozen at -70°C ± 10°C.

In the blind passage, all of the uninoculated control suckling mice, all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice and seven of the twenty suckling mice inoculated with the homogenate of the test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. Two of the suckling mice inoculated with the homogenate of the test article inoculated suckling mice were found partially cannibalized; one on day 9 post-inoculation and one on day 10 post-inoculation. Four additional suckling mice in the test article homogenate group were moribund day 10 post-inoculation and were sacrificed. Necropsy was performed. Four of the test article homogenate inoculated suckling mice were found dead day 12 post-inoculation. One of the test article homogenate inoculated suckling mice appeared lethargic day 13 post-inoculation and was found partially cannibalized day 14 post-inoculation. Of the remaining suckling mice, two appeared lethargic on day 14 post-inoculation. It was requested by the sponsor that no histopathologic examination be performed on the surviving, dead, or moribund suckling mice. The death of the suckling mice was most likely caused by the specific virus in the product, and was not an unexpected response to the intracranial inoculation of suckling mice with this product.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7, was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive due to the lethal effect of the specific virus in the product, on the suckling mice.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 10/23/90

Mary D. Whiteman
Study Director

Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

SAA = Same as above
i.c. = Intracranial
i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

	Adult Mice[a]	Suckling Mice[b] Primary Inoculation	Suckling Mice[b] Blind Passage
Test Article	20/20	20/20	9/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control[c]			10/10

[a] Number of surviving, animals after 28 days/Number of animals inoculated.

[b] In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

[c] In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.
Adult Mice	Test Article	10631	Normal		
		10632	Normal		
		10633	Normal		
		10634	Normal		
		10635	Normal		
		10636	Normal		
		10637	Normal		
		10638	Normal		
		10639	Normal		
		10640	Normal		
		10641	Normal		
		10642	Normal		
		10643	Normal		
		10644	Normal		
		10645	Normal		
		10646	Normal		
		10647	Normal		
		10648	Normal		
		10649	Normal		
		10650	Normal		
	Uninoc-ulated Control	10621	Normal		
		10622	Normal		
		10623	Normal		
		10624	Normal		
		10625	Normal		
		10626	Normal		
		10627	Normal		
		10628	Normal		
		10629	Normal		
		10630	Normal		

TABLE 3 (Cont.)
Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
Suckling[b] Mice (Primary Inoculation)	Test Article	SM1 (10)	Normal		
		(10)[g]		14	
		SM2 (10)	Normal		
	Uninoculated Control	SM3 (10)	Normal		
(Blind Passage)	Test Article Homogenate	SM1 (10)	Normal		
		(9)[c]		9	9
		(8)[c]		10	10
		(4)[d]		10	10
		(0)[e]		12	12
		SM2 (10)	Normal		
		(9)[f]		13	14
		(9)[h]		14	
	Uninoculated Control Homogenate	SM3 (10)	Normal		
	Uninoculated Control	SM4 (10)	Normal		

[a] Ten suckling mice inoculated per cage.
[b] Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.
[c] One suckling mouse was found partially cannibalized.
[d] Four suckling mice appeared moribund and were sacrificed. Necropsy was performed.
[e] Four suckling mice were found dead.
[f] One suckling mouse appeared runted and hunched day 13 post-inoculation and was found partially cannibalized day 14.
[g] One suckling mouse was lethargic and appeared to have a swollen head.
[h] Two suckling mice were lethargic and had hunched postures.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA360.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/23/90, TO MGMT 08/23/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 10/17/90 - 10/17/90, TO STUDY DIR 10/17/90, TO MGMT 10/25/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Dana H. Hamblen — Date: 10-25-90
Quality Assurance Unit

ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study No.: ZA361.005100

Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Final Report
For

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this assay is to attempt to detect the presence of adventitious agent(s) in the test article, pre-clarified bulk live virus vaccines and/or fluids, other than the specific virus in the product. The test article was inoculated into adult mice and suckling mice. The suckling mouse portion of the assay included a subpassage of homogenized tissue after 14 days into a new group of suckling mice, followed by an additional 14 day observation period.

No evidence of contamination with adventitious agents due to the test article Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive, due to the lethal effect of the specific virus in the product on the suckling mice.

ZA361.005100

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes inoculations of adult and suckling mice. The test is performed as described in CFR Title 21, Section 630.35 (a)(1)(2).

Adult mice are included in the assay to detect possible contamination of the test article with neurotropic or other viruses such as lymphocytic choriomeningitis virus. Suckling mice are used to detect Coxsackie or other viruses. All animals are observed for signs of illness and any that become sick or show any abnormalities are examined in an attempt to establish cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Mice and Suckling Mice

B. Study Number: ZA361.005100

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Mice

Suckling litters (Primary Inoculation): Tac:(SW)fBR, three adult females each with at least eight <24 hour old suckling pups,
Source: Taconic Farms, Germantown, New York

Suckling litters (Blind Passage): Tac(SW)fBR, six adult females each with ten <24 hour old suckling pups,
Source: Taconic Farms, Germantown, New York

Adult - HSD:ICR, Fifteen males and fifteen females, Body Weight range: 15-20 grams.
Source: Harlan Sprague Dawley, Frederick, Maryland

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/16/90

2. Lab Initiation Date: 07/24/90

3. Lab Completion Date: 08/28/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of

samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

With approval of the sponsor, a previously thawed test article sample which was frozen back and stored at -70°C ± 10°C was utilized in the inoculation of the suckling mouse portion of the assay. In addition, the sponsor requested that in the suckling mouse portion of the assay 1.3 ml of the test article be combined with 1.3 ml of the sponsor supplied antisera Den-4 #814669 Carec, SM5 of 11-21-82 and heated at 37°C for 90 minutes prior to inoculation of the suckling mice. This procedure was followed except that due to an insufficient volume of antisera, 1.2 ml of antisera was combined with 1.2 ml of the test article. This procedural change was approved by the sponsor. The remaining untreated sample was frozen back and was utilized along with a previously unthawed aliquot of the test article to inoculate the adult mouse portion of the assay.

A. Objective:

The study objective is to detect inapparent virus(es) that might be present in the test article, pre-clarified bulk live virus and/or fluids, other than the specific virus in the product.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Adult mice were ear-tagged but housed in groups according to inoculum type and sex. Suckling mice were not individually identified.

Suckling and adult mice were randomized according to SOP #OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. The adult mice were observed every working day for 28 days for clinical signs. In the suckling mouse portion of the assay, the animals were inoculated according to Table 1 and were then observed every working day for 14 days for clinical signs.

Note: After preparation of a 1:1 dilution of the remaining antisera Den-4 #814669 Carec, SM5 of 11-21-82 and the test article, the resulting volume was not sufficient to inoculate 10 suckling mice per group. After consultation with the sponsor, and with the sponsors approval, eight suckling mice were inoculated per group.

Fourteen days post-inoculation, all surviving suckling mice from each group were euthanized using cervical dislocation. Following euthanasia their skin and gastrointestine were removed, the carcasses cut into pieces and placed in a sterile pre-weighed bowl. After determining the weight of the entire group of mice from a cage, enough Hank's Balanced Salt Solution (HBSS) with gentamycin was added to make a 20% w/v suspension. The entire content of the bowl was then homogenized in a sterile blender, clarified by centrifugation, diluted 1:2 in HBSS and subsequently inoculated into a new group of suckling mice by the same routes and in the same volumes as the original group. These newly inoculated mice were observed for a period of fourteen days. Ten suckling mice were held as uninoculated controls and observed for fourteen days.

3. Animal Husbandry

a. All animals were fed the following diet ad libitum:

Mice - autoclavable chow.

b. Water was supplied ad libitum via fresh apples.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

IV. RESULTS

All adult mice inoculated with the test article and all uninoculated control adult mice remained normal and healthy for the twenty-eight day observation period.

All of the uninoculated control suckling mice and six of the sixteen test article inoculated suckling mice appeared normal and healthy for the 14 day observation period. One of the test article inoculated suckling mice was missing and presumed cannibalized day one post-inoculation. On day 13 post-inoculation, five of the test article inoculated suckling mice appeared moribund and were sacrificed and homogenized for passage into new groups of suckling mice. Four of the test article inoculated suckling mice were found dead; three on day 13 and one on day 14 post-inoculation. Necropsy and histopathology were performed on the dead suckling mice. The cause of death of these animal could not be determined due to the degree of autolysis in the tissues examined. (See Pathology Report in Appendix.)

In the blind passage, all of the uninoculated control suckling mice and all of the suckling mice inoculated with the homogenate of the uninoculated control suckling mice appeared normal and healthy for the 14 day observation period. One of the test article homogenate inoculated suckling mice was missing and presumed cannibalized day 9 post-inoculation. By day 10 post-inoculation, all but one of the remaining suckling mice appeared lethargic and hunched and were staggering. On day 11 post-inoculation, one test article homogenate inoculated suckling mouse was found dead. Eight test article homogenate inoculated suckling mice were found dead day 12 post-inoculation. Two of the dead suckling mice were partially cannibalized. On day 13 post-inoculation, of the ten remaining test article homogenate inoculated suckling mice, five were found dead, one was found partially cannibalized and four were moribund. Two of the moribund suckling mice inoculated with the test article homogenate, were sacrificed. Necropsy was performed. On day 14 post-inoculation, the remaining two suckling mice were found dead.

Of the twenty suckling mice inoculated with the homogenates of the test article inoculated mice which were moribund day 13 post-inoculation, eight appeared normal and healthy for the 14 day observation period. Two of the suckling mice inoculated with the homogenate of the test article moribund sucklings were missing and presumed cannibalized day 1 post-inoculation and one was missing and presumed cannibalized day 4 post-inoculation. Of the seventeen remaining suckling mice inoculated with the homogenate of the moribund test article suckling mice, on day 13 post-inoculation, two were found partially cannibalized, four were moribund and one was runted and lethargic. One of the four moribund suckling mice and the runted suckling mouse were sacrificed. Necropsy was performed. On day 14 post-inoculation, three suckling mice were found dead and two were moribund.

It was requested by the sponsor that no histopathologic examination be performed on the dead, sacrificed or moribund suckling mice. The death of the suckling mice was most likely caused by the specific virus in the product and was not an unexpected response to the intracranial inoculation of suckling mice with this product.

See Tables 2 and 3 for a summary of the data.

V. CONCLUSIONS

No evidence of contamination with adventitious agents due to the test article, Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7, was observed in the adult mouse portion of the assay. The results of the suckling mouse portion of the assay were inconclusive due to the lethal effect of the specific virus in the product on the suckling mice.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice Regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman
Study Director

10/24/90
Date

TABLE 1

Group #	Number of Animals	Sex	Species	Route(s) of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
SM 1	1	female	mouse (lactating)	None	None	None	None
	+ 10	various	Mouse (suckling)	i.p. i.c.	0.1 ml 0.01 ml	test article test article	Observed for illness after 14 days passage a single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice onto at least 10 additional suckling mice. Use same routes and volumes as original.
SM 2	SAA*	SAA	SAA	SAA	SAA	SAA	SAA
SM 3	SAA	SAA	SAA	None	None	uninoc control	SAA
AM 1	5	male	mouse	i.p. i.c.	0.5 ml 0.03 ml	test article	Observe for illness
AM 2	5	male	SAA	SAA	SAA	SAA	SAA
AM 3	5	female	SAA	SAA	SAA	SAA	SAA
AM 4	5	female	SAA	SAA	SAA	SAA	SAA
AM 5	5	male	SAA	None	None	uninoc control	SAA
AM 6	5	female	SAA	None	None	SAA	SAA

SAA = Same as above
i.c. = Intracranial
i.p. = Intraperitoneal

TABLE 2

Survival Summary
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

	Adult Mice[a]	Suckling Mice[b] Primary Inoculation	Suckling Mice[b] Blind Passage
Test Article	20/20	6/16	0/20
Uninoculated Control	10/10	10/10	10/10
Uninoculated Control[c]			10/10
Test Article Homogenate of Moribund Sucklings			10/20

[a] Number of surviving, animals after 28 days/Number of animals inoculated.

[b] In the suckling mice portion of the assay, animals are inoculated and observed for 14 days. On day 14 post-inoculation a homogenate is prepared from the surviving sucklings from each group. This homogenate was used to inoculate another group of suckling mice which were observed for an additional 14 days. The number of surviving sucklings/number of animals inoculated is presented.

[c] In the blind passage of the suckling mouse portion of the assay an uninoculated control group was held with the blind passage animals inoculated with the homogenate of the test article and the homogenate of the uninoculated control group from the primary inoculation.

TABLE 3

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.
Adult Mice	Test Article	10651	Normal		
		10652	Normal		
		10653	Normal		
		10654	Normal		
		10655	Normal		
		10656	Normal		
		10657	Normal		
		10658	Normal		
		10659	Normal		
		10660	Normal		
		10661	Normal		
		10662	Normal		
		10663	Normal		
		10664	Normal		
		10665	Normal		
		10666	Normal		
		10667	Normal		
		10668	Normal		
		10669	Normal		
		10670	Normal		
	Uninoc-ulated Control	10621	Normal		
		10622	Normal		
		10623	Normal		
		10624	Normal		
		10625	Normal		
		10626	Normal		
		10627	Normal		
		10628	Normal		
		10629	Normal		
		10630	Normal		

TABLE 3 (Cont.)

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
Suckling[b] Mice (Primary Inoculation)	Test Article	SM1 (8)	Normal		
		(6)[f]		13	13
		(3)[g]		13	13
		(2)[d]		14	14
		SM2 (8)	Normal		
		(7)[c]		1	1
		(6)[d]		13	13
		(4)[e]		13	13
	Uninoculated Control	SM3 (10)	Normal		

[a] Eight to ten suckling mice inoculated per cage.
[b] Surviving suckling mice from primary inoculation were sacrificed on day 14 for preparation of blind passage tissue homogenate.
[c] One suckling mouse missing and presumed cannibalized.
[d] One suckling mouse found dead and sent for necropsy and histopathology.
[e] Two moribund suckling mice were homogenized for passage.
[f] Two suckling mice found dead and sent for necropsy and histopathology.
[g] Three moribund suckling mice were homogenized for passage.

TABLE 3 (Cont.)
Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Number/ Cage[a]	Clinical Signs	Day of Onset (Post-Inoc.)	Day of Death/ Sacrifice (Post-Inoc.)
(Blind Passage)	Test Article Homo-genate	SM1 (10)	Normal		
		(10)[h]		10	
		(9)[i]		11	11
		(4)[j]		12	12
		(0)[k]		13	13
		SM2 (10)	Normal		
		(9)[c]		9	9
		(9)[l]		10	
		(6)[m]		12	12
		(2)[n]		13	13
		(0)[o]		14	14
	Uninocu-lated Control Homo-genate	SM3 (10)	Normal		
	Uninocu-lated Control	SM4 (10)	Normal		
	Test Article Homogen-ate of Moribund Mice	SM5 (10)	Normal		
		(8)[p]		1	1
		(5)[q]		13	13
		(2)[r]		14	14
		SM6 (10)	Normal		
		(9)[c]		4	4
		(8)[s]		13	13

[h] All ten sucklings were lethargic, hunched and were staggering.
[i] One suckling mouse was found dead.
[j] Five suckling mice were found dead.
[k] Three suckling mice were found dead; remaining suckling was moribund and was sacrificed and sent for necropsy.
[l] Eight suckling mice were lethargic, hunched, and were staggering.
[m] Three suckling mice were found dead; of the three, two were partially cannibalized.
[n] Two suckling mice were found dead and one was found partially cannibalized; three were moribund and one of the three moribund mice was sacrificed and sent for necropsy.
[o] Two suckling mice were found dead.
[p] Two suckling mice were missing and presumed cannibalized.
[q] Two suckling mice found partially cannibalized; four appeared moribund; one appeared lethargic and runted; one of the four moribund sucklings was sacrificed and sent for necropsy.
[r] Three suckling mice were found dead; remaining two sucklings appeared moribund.
[s] One suckling mouse appeared lethargic and slightly runted, and was sacrificed and sent for necropsy.

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT MICE AND SUCKLING MICE

Study Number: ZA361.005100

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/19/90 - 07/19/90, TO STUDY DIR 07/19/90, TO MGMT 07/19/90
PHASES: PROTOCOL REVIEW

INSPECT ON 07/24/90 - 07/24/90, TO STUDY DIR 07/24/90, TO MGMT 07/24/90
PHASES: ADMINISTRATION OF THE TEST ARTICLE
TO THE TEST SYSTEM

INSPECT ON 10/17/90 - 10/17/90, TO STUDY DIR 10/17/90, TO MGMT 10/25/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Dana H. Hamblen — Date: 10-25-90
Quality Assurance Unit

IX. APPENDIX

PAI Pathology Associates, Inc.

Suite I
15 Worman's Mill Court
Frederick, Maryland 21701
Phone: (301) 663-1644
FAX: (301) 663-8994

PATHOLOGY REPORT

CAHS 2187		**DATE RECEIVED:**	8/6/90
SOURCE:	Biotech Services ZA361.005100	**DATE NECROPSIED:**	8/7/90
SPECIES:	Mouse	**DATE REPORTED:**	8/28/90

RESULTS:

Organs examined microscopically: Nose, eyes, ears, trachea, brain, heart, kidney, lung, liver, spleen, ileum, cecum, colon, gross lesions.

2187-1 Suckling Test Article Cage 6 5g.

Gross: Animal received dead.
Abdominal organs partially autolyzed.
Brain tissue soft.
Red color over top of nasal cavity.

Microscopic: All tissues examined -- Autolysis

2187-2 Suckling Test Article Cage 6 4g.

Gross: Animal received dead.
Abdominal organs partially autolyzed.
Brain tissue soft and dark red in color.

Microscopic: All tissues examined -- Autolysis

2187-3 Suckling Test Article Cage 7 5g.

Gross: Animal received dead.
Organs partially autolyzed.
Brain tissue soft and darkly colored red.

Microscopic: All tissues examined -- Autolysis

PATHOLOGY REPORT

CAHS 2187		**DATE RECEIVED:**	8/7/90
SOURCE:	Biotech Services ZA361.005100	**DATE NECROPSIED:**	8/8/90
SPECIES:	Mouse	**DATE REPORTED:**	8/28/90

RESULTS:

2187-4	Suckling	Test Article	Cage 6	5g.

Gross: Animal received dead.
Abdominal tissues darkly colored.
All tissues partially autolyzed.
Brain tissue soft .

Microscopic: All tissues examined -- Autolysis

COMMENT: The cause of death cannot be determined due to the marked autolysis in the examined tissues.

Lynda L. Pippin DVM
Lynda L. Pippin, DVM

APPENDIX

D

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA359.005102

Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this test is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agent(s). The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Guinea Pigs

B. Study Number: ZA359.005102

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Guinea Pigs
Hartley Albino
6 adult females,
Body weight range: 350-400 g
Source: Hazleton Research Animals
Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agents that might be present in the test article.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

3. Animal Husbandry

a. Animals were fed Ralston Purina Certified Guinea Pig Chow.

b. Water was supplied ad libitum via water bottles. Water was disinfected with 7 ppm chlorine.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post-Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through day 42 post-inoculations. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the uninoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman
Study Director

9/13/90
Date

TABLE 2

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-6, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.	Range of Body Temp in °C D-21 to D-42
Guinea Pig	Test Article	10421	Normal			37.2 - 38.7
		10422	Normal			38.1 - 38.6
		10423	Normal			38.1 - 38.6
	Uninoc-ulated Control	10424	Normal			38.1 - 38.6
		10425	Normal			37.5 - 38.7
		10426	Normal			38.1 - 38.5

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA359.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/14/90 - 08/14/90, TO STUDY DIR 08/14/90, TO MGMT 08/21/90
PHASES: RECTAL TEMPERATURE DETERMINATION

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan McGowan — 9/14/90

Joan M. McGowan
Quality Assurance Unit

Date

VIII. APPENDIX

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

PATHOLOGY REPORT

CAHS-2190 **DATE RECEIVED:** 08/23/90

SOURCE: Biotech Services **DATE NECROPSIED:** 08/23/90
ZA356.005102 ZA357.005102
ZA358.005102 ZA359.005102 **DATE REPORTED:** 09/06/90
ZA360.005102 ZA361.005102

SPECIES: Guinea Pig
Results of gross examination according to SOP #865.201.

ZA356.005102

2190-1 (10401) Test Article 563.9 g
Gross: No lesions found

2190-2 (10402) Test Article 684.2 g
Gross: No lesions found

2190-3 (10403) Test Article 614.8 g
Gross: No lesions found

2190-4 (10404) Control 614.4 g
Gross: No lesions found

2190-5 (10405) Control 673.3 g
Gross: No lesions found

2190-6 (10406) Control 646.2 g
Gross: No lesions found

ZA357.005102

2190-7 (10407) Test Article 629.0 g
Gross: No lesions found

2190-8 (10408) Test Article 583.6 g
Gross: No lesions found

2190-9 (10409) Test Article 528.1 g
Gross: No lesions found

ZA358.005102

2190-10 (10410) Test Article 636.3 g
Gross: No lesions found

2190-11 (10411) Test Article 490.5 g
Gross: Retroperitoneal abscess (4x6 cm) and peritonitis

2190-12 (10412) Test Article 595.5 g
Gross: No lesions found

ZA359.005102

2190-13 (10421) Test Article 605.3 g
Gross: No lesions found

2190-14 (10422) Test Article 631.8 g
Gross: No lesions found

2190-15 (10423) Test Article 547.4 g
Gross: No lesions found

2190-16 (10424) Control 651.5 g
Gross: No lesions found

2190-17 (10425) Control 623.6 g
Gross: No lesions found

2190-18 (10426) Control 615.9 g
Gross: No lesions found

ZA360.005102

2190-19 (10427) Test Article 561.8 g
Gross: No lesions found

2190-20 (10428) Test Article 594.6 g
Gross: No lesions found

2190-21 (10429) Test Article 598.4 g
Gross: No lesions found

ZA361.005102

2190-22 (10430) Test Article 559.8 g
Gross: No lesions found

2190-23 (10431) Test Article 547.9 g
Gross: No lesions found

2190-24 (10432) Test Article 624.8 g
Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA360.005102

Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this test is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7.

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agents. The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Guinea Pigs

B. Study Number: ZA360.005102

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Guinea Pigs
Hartley Albino
6 adult females,
Body weight range: 350-400 g

Source: Hazleton Research Animals
Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agents that might be present in the test article.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

3. Animal Husbandry

a. Animals were fed Ralston Purina Certified Guinea Pig Chow.

b. Water was supplied ad libitum via water bottles. Water was disinfected with 7 ppm chlorine.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post-Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections during the 21 day recording period from day 21 through day 42 post-inoculation. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the inoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/13/90

Mary D. Whiteman
Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-10, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.	Range of Body Temp in °C D-21 to D-42
Guinea Pig	Test Article	10427	Normal			38.0 - 38.6
		10428	Normal			38.0 - 38.5
		10429	Normal			38.2 - 38.8
	Uninoc-ulated Control	10424	Normal			38.1 - 38.6
		10425	Normal			37.5 - 38.8
		10426	Normal			38.1 - 38.5

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA360.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90
PHASES: PROTOCOL REVIEW

INSPECT ON 07/30/90 - 07/30/90, TO STUDY DIR 07/30/90, TO MGMT 08/06/90
PHASES: ANIMAL OBSERVATIONS

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan — 9/14/90

Joan M. McGowan
Quality Assurance Unit

Date

VIII. APPENDIX

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

PATHOLOGY REPORT

CAHS-2190 **DATE RECEIVED:** 08/23/90

SOURCE: Biotech Services **DATE NECROPSIED:** 08/23/90
ZA356.005102 ZA357.005102
ZA358.005102 ZA359.005102 **DATE REPORTED:** 09/06/90
ZA360.005102 ZA361.005102

SPECIES: Guinea Pig
Results of gross examination according to SOP #865.201.

ZA356.005102

2190-1 (10401) Test Article 563.9 g
Gross: No lesions found

2190-2 (10402) Test Article 684.2 g
Gross: No lesions found

2190-3 (10403) Test Article 614.8 g
Gross: No lesions found

2190-4 (10404) Control 614.4 g
Gross: No lesions found

2190-5 (10405) Control 673.3 g
Gross: No lesions found

2190-6 (10406) Control 646.2 g
Gross: No lesions found

ZA357.005102

2190-7 (10407) Test Article 629.0 g
Gross: No lesions found

2190-8 (10408) Test Article 583.6 g
Gross: No lesions found

2190-9 (10409) Test Article 528.1 g
Gross: No lesions found

ZA358.005102

2190-10 (10410) Test Article 636.3 g
Gross: No lesions found

2190-11 (10411) Test Article 490.5 g
Gross: Retroperitoneal abscess (4x6 cm) and peritonitis

2190-12 (10412) Test Article 595.5 g
Gross: No lesions found

ZA359.005102

2190-13 (10421) Test Article 605.3 g
Gross: No lesions found

2190-14 (10422) Test Article 631.8 g
Gross: No lesions found

2190-15 (10423) Test Article 547.4 g
Gross: No lesions found

2190-16 (10424) Control 651.5 g
Gross: No lesions found

2190-17 (10425) Control 623.6 g
Gross: No lesions found

2190-18 (10426) Control 615.9 g
Gross: No lesions found

ZA360.005102

2190-19 (10427) Test Article 561.8 g
Gross: No lesions found

2190-20 (10428) Test Article 594.6 g
Gross: No lesions found

2190-21 (10429) Test Article 598.4 g
Gross: No lesions found

ZA361.005102

2190-22 (10430) Test Article 559.8 g
Gross: No lesions found

2190-23 (10431) Test Article 547.9 g
Gross: No lesions found

2190-24 (10432) Test Article 624.8 g
Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study No.: ZA361.005102

Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Final Report

Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

By
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

TABLE OF CONTENTS

SUMMARY

The purpose of this test is to attempt to detect the presence of adventitious agent(s) in the test article, preclarified bulk live virus and/or fluids, other than the specific virus in the product, by the inoculation and observation of guinea pigs.

No evidence of contamination with adventitious agents was observed due to the test article Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7.

ZA361.005102

I. INTRODUCTION

It is the purpose of this study to detect the presence of latent or inapparent adventitious agents. The experimental design utilizes the inoculation of adult guinea pigs. The test is performed as described in CFR Title 21, Section 630.16(a)(4).

Adult guinea pigs are used in this assay to detect possible contamination of the test article with M. tuberculosis or other adventitious agent(s). Animals are examined for signs of illness, and any that become sick or show any abnormalities are examined in an attempt to determine the cause of illness or death.

II. STUDY INFORMATION

A. Title: Animal Safety Test in Adult Guinea Pigs

B. Study Number: ZA361.005102

C. Test Article: Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7 was received at Microbiological Associates, Inc. on 07/06/90. Determination of the stability, purity and concentration of the test article is the responsibility of the sponsor.

D. Medium Test Article: none

E. Control Articles:

1. Positive Control: none

2. Negative Control: none

3. Vehicle Control: none

F. Test System: Guinea Pigs
Hartley Albino
6 adult females,
Body weight range: 350-400 g
Source: Hazleton Research Animals
Denver, Pennsylvania

G. Sponsor: Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22102

Authorized Representative: Dr. Louis Potash

H. Testing Facility: Biotechnology Services Department
Microbiological Associates, Inc.
Life Sciences Center
9900 Blackwell Road
Rockville, Maryland 20850

Animal Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

I. Personnel:

1. Study Director: Mary D. Whiteman

2. Associate
Study Director: Janet Luczak, M.T.(ASCP), M.G.A.

J. Schedule:

1. Study Initiation Date: 07/11/90

2. Lab Initiation Date: 07/12/90

3. Lab Completion Date: 09/06/90

4. Study Completion Date: See Study Director's Signature Date, in the "Approvals" Section.

K. Raw Data, Records and Test Article Samples:

All raw data, records, protocol and all report copies will be maintained by the testing facility, Regulatory Affairs/Quality Assurance Department. Retention of samples of the test article is the responsibility of the sponsor.

L. Archive:

Study records will be archived by the Regulatory Affairs/Quality Assurance Department, Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850.

III. PROCEDURES

A. Objective:

The study objective is to detect adventitious agent(s) that might be present in the test article.

B. Methods:

1. Test System Identification and Randomization

Each animal cage was assigned a number and labelled as "test article" or "uninoculated control". Animals were housed separately and were identified by ear tags.

The guinea pigs were randomized according to SOP OPBT0213.

2. Animal Inoculation with Test Article

Animals were inoculated according to Table 1. Three were held as uninoculated controls. All animals were observed every working day of the 42 day test period for death or clinical signs of illness or distress. Beginning day 21 post inoculation, rectal temperatures were recorded through day 42 post-inoculation. All remaining guinea pigs were sacrificed. Gross pathological examinations were performed on all guinea pigs.

3. Animal Husbandry

a. Animals were fed Ralston Purina Certified Guinea Pig Chow.

b. Water was supplied ad libitum via water bottles. Water was disinfected with 7 ppm chlorine.

c. Bedding - Bed-o-Cobs, Anderson Cob Mills. Cages were changed as necessary, usually twice per week.

d. Animal facilities are accredited by the American Association for Accreditation of Laboratory Animal Care.

ZA361.005102

TABLE 1

Group #	No. of Animals	Routes of Inoculation	Volume of Inoculum	Inoculum	Treatment after Inoculation
1	1	I.C. I.P.	0.1 ml 5.0 ml	Test Article	Observe for Illness Record Rectal Temp. On Days 21-42 Post-Inoculation
2	1	SAA	SAA	SAA	SAA
3	1	SAA	SAA	SAA	SAA
4	1	None	None	Uninoc Control	SAA
5	1	SAA	SAA	SAA	SAA
6	1	SAA	SAA	SAA	SAA

SAA = Same As Above
I.C. = Intracranial
I.P. = Intraperitoneal

IV. RESULTS

All of the uninoculated control guinea pigs and all of the test article inoculated guinea pigs appeared normal and healthy throughout the 42 day observation period. See Table 2 for a summary of the data.

None of the uninoculated control guinea pigs and none of the test article inoculated guinea pigs had significant temperature rises indicative of either viral or bacterial infections, during the 21 day recording period from day 21 through day 42 post-inoculation. See Table 2 for a summary of the data.

At examination on day 42 for gross pathology, no lesions were found in the uninoculated control or test article guinea pigs. (See Pathology Report in Appendix.)

V. CONCLUSIONS

No evidence of contamination with adventitious agent(s) due to the test article, Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7, was observed.

VI. APPROVALS

This study met the criteria for a valid test and was performed in compliance with the requirements of the Food and Drug Administration's Good Laboratory Practice regulations as found in Title 21 CFR Part 58.

Mary D. Whiteman — 9/13/90

Mary D. Whiteman
Study Director

Date

TABLE 2

Summary of Daily Observations
for Dengue-4 (#341750) Prod Seed, PDK-15, FRhL-2/d7

Animal Species	Inoculum	Animal Number	Clinical Signs	Day of Onset (Post-inoc.)	Day of Death/ Sacrifice (Post-inoc.	Range of Body Temp in °C D-21 to D-42
Guinea Pig	Test Article	10430	Normal			38.1 - 38.8
		10431	Normal			38.1 - 38.7
		10432	Normal			38.1 - 38.6
	Uninoc-ulated Control	10424	Normal			38.1 - 38.6
		10425	Normal			37.5 - 38.8
		10426	Normal			38.1 - 38.5

QUALITY ASSURANCE STATEMENT

Study Title: ANIMAL SAFETY TEST IN ADULT GUINEA PIGS

Study Number: ZA361.005102

Study Director: Mary D. Whiteman

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), and the OECD Guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 07/16/90 - 07/16/90, TO STUDY DIR 07/16/90, TO MGMT 07/16/90
PHASES: PROTOCOL REVIEW

INSPECT ON 08/23/90 - 08/23/90, TO STUDY DIR 08/24/90, TO MGMT 08/27/90
PHASES: EXAM. OF ABDOMINAL AND THORACIC VISCERA AT DAY 42 POST-INOCULATION FOR OBVIOUS OR SUGGESTIVE ABNORMALITIES

INSPECT ON 09/12/90 - 09/12/90, TO STUDY DIR 09/12/90, TO MGMT 09/14/90
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan Mc Gowan — 9/14/90

Joan M. McGowan
Quality Assurance Unit

Date

VIII. APPENDIX

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 90-8793

PATHOLOGY REPORT

CAHS-2190 **DATE RECEIVED:** 08/23/90

SOURCE: Biotech Services **DATE NECROPSIED:** 08/23/90
ZA356.005102 ZA357.005102
ZA358.005102 ZA359.005102 **DATE REPORTED:** 09/06/90
ZA360.005102 ZA361.005102

SPECIES: Guinea Pig
Results of gross examination according to SOP #865.201.

ZA356.005102

2190-1 (10401) Test Article 563.9 g
Gross: No lesions found

2190-2 (10402) Test Article 684.2 g
Gross: No lesions found

2190-3 (10403) Test Article 614.8 g
Gross: No lesions found

2190-4 (10404) Control 614.4 g
Gross: No lesions found

2190-5 (10405) Control 673.3 g
Gross: No lesions found

2190-6 (10406) Control 646.2 g
Gross: No lesions found

ZA357.005102

2190-7 (10407) Test Article 629.0 g
Gross: No lesions found

2190-8 (10408) Test Article 583.6 g
Gross: No lesions found

2190-9 (10409) Test Article 528.1 g
Gross: No lesions found

ZA358.005102

2190-10 (10410) Test Article 636.3 g
Gross: No lesions found

2190-11 (10411) Test Article 490.5 g
Gross: Retroperitoneal abscess (4x6 cm) and peritonitis

2190-12 (10412) Test Article 595.5 g
Gross: No lesions found

ZA359.005102

2190-13 (10421) Test Article 605.3 g
Gross: No lesions found

2190-14 (10422) Test Article 631.8 g
Gross: No lesions found

2190-15 (10423) Test Article 547.4 g
Gross: No lesions found

2190-16 (10424) Control 651.5 g
Gross: No lesions found

2190-17 (10425) Control 623.6 g
Gross: No lesions found

2190-18 (10426) Control 615.9 g
Gross: No lesions found

ZA360.005102

2190-19 (10427) Test Article 561.8 g
Gross: No lesions found

2190-20 (10428) Test Article 594.6 g
Gross: No lesions found

2190-21 (10429) Test Article 598.4 g
Gross: No lesions found

ZA361.005102

2190-22 (10430) Test Article 559.8 g
Gross: No lesions found

2190-23 (10431) Test Article 547.9 g
Gross: No lesions found

2190-24 (10432) Test Article 624.8 g
Gross: No lesions found

COMMENT: The retroperitoneal abscess in guinea pig #2190-11 is believed to be due to a rectal perforation (no longer visible) caused by insertion of a temperature measuring probe. This is a common lesion in guinea pigs in which the probe is inserted on multiple occasions.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

ATTACHMENT A

MICROBIOLOGICAL ASSOCIATES INC.

CORPORATE OFFICES
Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Telex 908793 • Fax (301) 738-1036

BETHESDA LABORATORIES
5221 River Road • Bethesda • Maryland 20816
(301) 654-3400 • Telex 908793
Fax (301) 654-6916

December 17, 1990

Dr. Louis Potash
Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Road
McLean, VA 22101

Dear Dr. Potash,

Microbiological Associates, Inc. is an AAALAC accredited animal facility, and all studies are performed in accordance with the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23.

Sincerely,

Mary D. Whiteman

Mary D. Whiteman
Study Director, In Vivo Assays
Biotechnology Division

ATTACHMENT B

PRI
Program Resources, Inc.
Biomedical Services Division
7655 Old Springhouse Rd.
McLean, VA 22102 • (703) 506-0190
FAX (703) 506-0194

May 14, 1991

TO: Mr. Donald Holzworth, Vice President
Dr. Louis Potash, Study Director

FROM: James R. Plautz
Sr. QA Advisor

RE: GLP Compliance Audit of Final Reports for Safety Testing of Dengue Virus Type 1 and Type 4

On April 14, 1991 a complete audit for GLP compliance (21 CFR, Part 58) was conducted for the subject final reports and their respective raw data.

Our complete findings indicate that the studies were conducted under the guidance of the referenced Standard Operating Procedures (SOPs), the variations from the SOPs had no apparent effect on study outcome, and that the final report for each study is substantiated by the raw data.

Animal safety testing was conducted and reported separately from these final reports.

James R. Plautz
May 14, 1991